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DEVELOPMENT AND EVALUATION OF REPRODUCTIVE AND DEVELOPMENT TOXICITY TESTS FOR ASSESSING THE HAZARDS OF ENVIRONMENTAL CONTAMINANTS

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13. ABSTRACT *(Maximum 200 words)*
A subsurface spill of JP-4 jet fuel at Eglin Air Force Base (AFB), FL, was remediated using nitrate application. The Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) was used before, during, and after remediation to detect whether toxicity had been altered. Reproductive toxicity tests were also performed using adult male and female *Xenpous laevis* on soil samples. FETAX endpoints were the 96-hour LC50 and 96-hour EC50 (malformation) and growth. Male endpoints were sperm number, morphology, percent motile, and sperm speed. Female enpoints were egg weight, percent normal, percent fertilized, and percent normally cleaving. Offspring from matings of treated animals were reared for 96 hour and the mortality, malformation, and growth of the embryos were assessed. The developmental toxicity of JP-4 using FETAX was also established. Results from the Eglin AFB site suggested that JP-4 was developmentally toxic. The direct exposure method was a superior method to aqueous extraction or supercritical fluid extraction for embryos. Relatively high levels of developmental toxicity and some reproductive toxicity were present in pre-remediation soil samples. Post-remediation data suggested that toxicity was probably reduced in both the nitrate and control cells.

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PREFACE

This report was prepared by the Department of Zoology, Oklahoma State University, Stillwater, OK 74078, for the Armstrong Laboratory Environics Directorate (AL/EQ), Suite 2, 139 Barnes Drive, Tyndall Air Force Base, Florida 32403-5319.

A subsurface spill of JP-4 jet fuel at Eglin Air Force Base (AFB), FL, was remediated using nitrate application. The Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) was used before, during, and after remediation to detect whether toxicity had been altered. Reproductive toxicity tests were also performed using adult male and female *Xenpous laevis* on soil samples. FETAX endpoints were the 96-hour LC50 and 96-hour EC50(malformation) and growth. Male endpoints were sperm number, morphology, percent motile, and sperm speed. Female endpoints were egg weight, percent normal, percent fertilized, and percent normally cleaving. Offspring from matings of treated animals were reared for 96 hour and the mortality, malformation, and growth of the embryos were assessed. The developmental toxicity of JP-4 using FETAX was also established. Results from the Eglin AFB site suggested that JP-4 was developmentally toxic. The direct exposure method was a superior method to aqueous extraction or supercritical fluid extraction for embryos. Relatively high levels of developmental toxicity and some reproductive toxicity were present in pre-remediation soil samples. Post-remediation data suggested that toxicity was probably reduced in both the nitrate and control cells.

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This project was part of a larger research effort, coordinated by Dr. Steve Hutchins and funded by the US EPA and the Armstrong Laboratory, Environics Directorate, Tyndall AFB, FL (Alison Thomas, Project Officer) on bioremediation of fuel-contaminated aquifers using alternate electron acceptors. Samples were distributed to Oklahoma State University to evaluate the efficacy of bioremediation.

I would like to thank technicians Corinne McMillen, Mendi Hull, and Amy Lewis for their help on this project. I would also like to thank graduate students James Rayburn and Betsy Schrock for their work. Lastly, thanks are due to Jason Sides who served as an undergraduate research student on this project.

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EXECUTIVE SUMMARY

A. OBJECTIVE

The project goal was to develop and evaluate reproductive and developmental toxicity tests using the gametes and embryos of the South African clawed frog *Xenopus laevis* with particular emphasis on assessing the toxicity of contaminated soils. The developmental toxicity test used was FETAX (Frog Embryo Teratogenesis Assay-*Xenopus)* which is an established developmental toxicity test employing frog embryos. The reproductive toxicity tests using *Xenopus* males and females were newly designed for this study. These tests were then used to monitor the progress of nitrate remediation efforts of a JP-4 spill site at Eglin Air Force Base, (AFB) Florida. Specific objectives were:

1. Develop and evaluate exposure methods which effectively delivered toxicants to adult frogs or embryos. Methods of adult exposure were the feeding of supercritical fluid extracts (SCFE) via earthworms or other food and direct exposure to the soil for 60 to 90 days. Methods of embryo exposure included culture of embryos on core samples from contaminated and uncontaminated sites, a simple water extraction procedure and exposure to SCFE using emulsification into an underlying bed of 2 percent agarose. How a contaminant is taken up by a living organism plays a major role in determining toxicity.

2. The endpoints of a new reproductive toxicity test were identified and evaluated. The toxicity of contaminants on gamete growth, development and ability of treated frog oocytes to develop following ovulation and fertilization were studied. The reproductive toxicity test allowed for the testing of potentially critical phases of the life cycle of any vertebrate organism. The contaminated soils were tested for reproductive toxicants using these new tests before and after nitrate remediation. We attempted to determine whether nitrate treatment of the test cells changed reproductive toxicity using the new tests.

3. Contaminated soils were tested using FETAX, during nitrate remediation.

B: BACKGROUND

Previous field work at the U.S. Coast Guard Facility in Traverse City, Michigan, showed that alkylbenzenes in an aquifer contaminated with JP-4 jet fuel can be degraded by the indigenous microorganisms under denitrifying conditions. However, the lack of a suitable control site precluded a direct assessment of the benefits of nitrate addition relative to infiltration recharge without nitrate amendments. Without such a comparison, the economics of nitrate-based bioremediation versus pump-and-treat methods could not be determined. Therefore, research was undertaken to better define the control parameters and provide a direct comparison through operation of a pilot nitrate remediation project at a JP-4 jet fuel-contaminated aquifer at Eglin AFB, FL.

The plan was to thoroughly analyze the site using environmental chemistry, and developmental and reproductive toxicity tests prior to remediation. The initial sampling at the Eglin site was conducted March 22-25, 1993. Toxicity tests conducted during this time period were termed pre remediation testing. Square test and control cells were marked off, sprinkler systems installed and nitrate treatment (NO_3-N) commenced on April 7, 1994 at a rate of 10 mg/L. The control cell was treated with water only throughout the study. Nitrate levels were increased to 15 to 20 mg/L on July 15, 1994. On August 19-30, 1994 interim core sampling was performed and toxicity tests were performed which were termed during remediation testing. Because lysimeter data from August sampling showed incomplete transfer of nitrate, plots were stripped and weed barriers installed. The final performance evaluation was conducted May 13-30, 1995 and termed post remediation toxicity testing.

FETAX has previously been used in testing toxicity in surface waters, ground waters and in sediments. FETAX can be used in human health hazard assessment when used in conjunction with a metabolic activation system (MAS) consisting of Aroclor 1254-induced rat liver microsomes to simulate mammalian metabolism. FETAX is immediately useful in ecotoxicology without the activation system. An ASTM defined protocol exists for FETAX and the assay has been validated using mammalian teratogens. The repeatability and reliability of the test has been established.

C. SCOPE

This document shows how new exposure methods were adapted for FETAX to allow for soil samples to be tested. Even non-polar jet fuel could be used in the assay. FETAX endpoints of mortality, malformation and growth inhibition generally correlated with the amount of TPH and BTEX in the soil. It was hoped that remediation would occur until the disappearance of reproductive and developmental toxicity from the soil even though traces remained. FETAX and the reproductive toxicity tests would help answer the question- "How clean is clean?" Section I is an introduction to the problem, a description of the goals and objectives of the research and background material. Section II describes the site and the methodology employed in remediation. Section III deals with the successful modification of FETAX and how developmental toxicity results correlated with TPH and BTEX concentrations. Evidence is presented that suggests a general site cleanup occurred although the statistical model did not provide such an assessment. Section IV covers the male reproductive toxicity test with endpoints that accounted for the success of gametogenesis and subsequent embryonic development. The female reproductive toxicity test with similar endpoints is presented in Section V. Section VI covers the general conlusions stating that FETAX was successful, the reproductive toxicity tests yielded some data and that a general cleanup was observed although toxicity was still present at the last sampling. Section VII covers recommendations for future studies. Specific tables and figures are included in the report.

D. METHODLOGY

Standard FETAX was used which entailed the continuous exposure of blastulae to toxicants for 96 hours. At the swimming larva stage, the percent dead, percent malformed and

length data were colleced and analyzed. Exposure regimens included direct exposure above the Eglin AFB soil in sealed jars, exposure in Petri dishes to aqueous extracts of soils and exposure to SCFE.

Both male and female tests involved exposing adults in water above contaminated soil and feeding adults contaminated food. Exposure periods were for 60 days. Endpoint data for gametogenesis and successful embryo growth and development were collected. These assays were used to test pre remediation, during remediation and post remediation soil samples from Eglin AFB. For a more detailed description of the specific procedures used in the reproductive toxicity testing, refer to that specific section of the report.

F. RESULTS

At the beginning of the project, direct exposure, aqueous extraction, and SCF-agarose methods were explored as possible exposure procedures for FETAX. The aqueous extraction technique was not suitable for the purposes of this particular study. The SCF procedure developed by the National Risk Management Research Laboratory (NRMRL) extracted far more toxicity from the soil than could be explained. Although tests were performed to determine whether toxicity was coming from the soil or other apparatus, it was not possible to discover the source of toxicity. It became apparent that the direct exposure technique was the best method of exposure. The primary disadvantage of this system was that it could not be used with the MAS used to assess human health hazards. However, preliminary investigations with JP-4 revealed little bioactivation or deactivation. Therefore, reasonable results could be obtained with direct exposure and no MAS.

Weathered and unweathered JP-4 were developmentally toxic. It caused severe malformations and it inhibited embryonic growth significantly. Although MAS failed to change the results to a large degree, some slight deactivation was observed. However, this may not have been statistically significant.

For adult exposure, we developed a direct exposure technique which allowed exposing of the animal to contaminated soils. In this experimental design, most absorption of contaminants was through the porous amphibian skin. There was little chance of the contaminant first being detoxified by the digestive system or the liver. Reproductive toxicity was obtained and adults were killed using this exposure method. Oral exposure was also explored, and the best results were seen when the SCFE was first injected into fairly large earthworms, and then offered to the frog as food.

The reproductive toxicity tests were new in design and untested. It became obvious after the first series of tests that methods which required the frogs to spawn were not reliable even when the numbers of breeders were increased. The best technique was to kill exposed males and dissect out the testis and perform studies on these organs. Results were always obtained using this method. Although adult toxicity was observed, some reduction in sperm counts and changes in sperm morphology were seen even at the end of remediation. Reduction in male and female reproductive toxicity was observed from pre to post remediation.

FETAX endpoints were correlated with TPH and BTEX concentrations measured in Eglin AFB soil. It must be remembered that the JP-4 at this site was not only weathered, but that other remediation attempts were performed in the past. Only carefully controlled laboratory studies can correlate TPH and BTEX measurements from freshly produced JP-4 with FETAX endpoints. It was readily apparent that high FETAX mortality and malformation was seen at the site of the spill while the control site away from the spill showed little mortality at most soil layers. Considerable developmental toxicity was seen in a large number of soil layers in both the control and nitrate-treated cells. The presence of the black mat to retard grass growth did not seem to speed remediation as judged by toxicity results.

When different soil layers are taken into account we derived the following order of toxicity when compared to site location:

Mortality: GZ>NC>CCC>NCC>CC>KC
Malformation: GZ>CCC>NC>NCC>CC>KC
Growth: GZ>NCC>NC>CCC>CC>KC

Where GZ=ground zero; NC= nitrate cell; NCC= nitrate cell with black mat; CC= control cell (water only); CCC= control cell (water only) with black mat and KC= control cell (remote location).

As expected, GZ was the untreated area where the spill occurred and the toxicity was highest there. Also as expected, KC was the lowest because this site was out of the spill area. There was a fairly mixed pattern of toxicity for all of the other sites, indicating cleanup despite nitrate application. Had toxicity uniformly increased due to nitrate treatment, then the NC series would be more toxic than the CC series. If remediation had worked as planned, the CC series should have been more toxic than the NC.

When the FETAX data was compared from pre to post remediation, cleanup was somewhat evident in both nitrate and control (water only) cells.

H. RECOMMENDATIONS

With modifications, FETAX proved it can be used for soil toxicity testing. Improvements should be made to the direct exposure method to allow use of MAS and to have better controls. More work needs to be done with statistical models for toxicity data. The utility of these techniques would be greatly improved with these modifications. The reproductive toxicity tests show promise, but more development work needs to be done before their routine use is acceptable.

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SECTION I

INTRODUCTION

A. OBJECTIVE

Previous field work at the U.S. Coast Guard Facility in Traverse City, Michigan, has shown that alkylbenzenes in an aquifer contaminated with JP-4 jet fuel can be degraded by the indigenous microorganisms under denitrifying conditions. However, the lack of a suitable control site precluded a direct assessment of the benefits of nitrate addition relative to infiltration recharge without nitrate amendments. Without such a comparison, the economics of nitrate-based bioremediation versus pump-and-treat methods could not be determined. The following research was therefore undertaken to better define the control parameters and provide a direct comparison through operation of a pilot project at a JP-4 jet fuel-contaminated aquifer at Eglin AFB, FL. The objectives of this research were as follows:

1. provide thorough site characterization to delineate contaminant distribution and microbial activity in the aquifer,
2. conduct field and laboratory tests to provide design parameters for construction and operation of the pilot system,
3. design, construct, and operate the pilot system to provide a direct comparison of the effects of recharge with and without nitrate amendments,
4. use core and water analyses to compare the extent of benzene, alkylbenzene, and JP-4 degradation in two treatment areas,
5. evaluate changes in microbial populations and sediment toxicity as a result of nitrate-based bioremediation, and
6. use FETAX as a developmental toxicity screening test to assess the success of nitrate-based remediation.
7. Male and female reproductive toxicity tests were developed and tested to augment FETAX. Both toxicity tests employ stages of the life cycle thought to be "weak links" in the life cycle of vertebrates.

The specific objectives for toxicity testing were:

1. Develop and evaluate exposure methods which effectively deliver toxicants to adult frogs and embryos. Methods of adult exposure were the feeding of SCFEs via earthworms or other food and direct exposure to the soil for 60 to 90 days. Methods of embryo exposure for FETAX included culture of embryos on core samples from contaminated and uncontaminated sites, a simple water extraction procedure proposed by the State of Washington for use in FETAX, and exposure to SCFEs using emulsification into an underlying bed of 2 percent agarose.

2. Identify and evaluate endpoints of a new reproductive toxicity test to assess the toxicity of contaminants on gamete growth, development and ability to fertilize, as well as the effects of toxicants on the ability of treated frog oocytes to develop following ovulation and

fertilization. The reproductive toxicity test had to allow for the testing of this critical phase of the life cycle. The contaminated soils were tested for reproductive toxicants using these new tests, before and after nitrate remediation, to determine whether nitrate treatment increased or decreased reproductive toxicity.

3. Test the contaminated soil using FETAX (an established developmental toxicity test employing frog embryos) before, during, and after nitrate remediation. Determine whether nitrate treatment to the test cells increased or decreased developmental toxicity as determined by FETAX.

B. BACKGROUND

1. Problems in Remediating Storage Tank Spills

Leaking underground storage tanks are a major source of ground water contamination by petroleum hydrocarbons. There are approximately 1 million underground tanks storing gasoline in the U.S., and there have been 270,000 confirmed releases reported in the last 6 years (OUST, 1994). Gasoline and other fuels contain benzene, toluene, ethylbenzene, and xylenes (collectively known as BTEX) which are hazardous compounds regulated by the U.S. Environmental Protection Agency (EPA, 1977). Although these aromatic hydrocarbons are relatively water-soluble, they are contained in the immiscible bulk fuel phase which serves as a slow-release mechanism for sustained ground water contamination. Pump-and-treat technology alone is economically impractical for renovating aquifers contaminated with bulk fuel, because the dynamics of immiscible fluid flow result in prohibitively long time periods for removal of the organic phase (Wilson and Conrad, 1984; Bouchard et al., 1989). In many cases, the problem is mitigated through the use of *in situ* aerobic bioremediation, which involves the addition of nutrients and oxygen to the contaminated areas so that the indigenous microbial populations can degrade the contaminants (Thomas et al., 1987; Lee et al., 1988; Atlas, 1991). Although aerobic bioremediation has been successfully applied (Raymond et al., 1978; Lee and Raymond, 1991; Bell and Hoffman, 1991), difficulties relating to aquifer plugging and oxygen mass transport are often encountered in inducing aerobic conditions by addition of oxygen or hydrogen peroxide to the subsurface environment (Wilson et al., 1986; Barker et al., 1987; Aggarwal et al., 1991).

Nitrate can also serve as an electron acceptor and results in anaerobic biodegradation of organic compounds via the processes of nitrate reduction and denitrification (Tiedje, 1988). Because nitrate is less expensive and more soluble than oxygen, it may be more economical to remediate fuel-contaminated aquifers using nitrate rather than oxygen. Several investigators have demonstrated that monoaromatic hydrocarbons can be degraded under denitrifying conditions. Zeyer et al. (1986) showed that toluene and *m*-xylene could be mineralized under denitrifying conditions in laboratory aquifer columns, and a pure culture was subsequently obtained with the same activity (Dolfing et al., 1990). The *m*-xylene-adapted microorganisms were unable to utilize benzene, ethylbenzene, and *o*- and *p*-xylene (Kuhn et al., 1988). Major et al. (1988), using aquifer material, observed biodegradation of benzene, toluene, and all three xylene isomers under denitrifying conditions. Hutchins et al. (1991a) found that toluene, ethylbenzene, xylenes, and 1,2,4-trimethylbenzene were degraded by aquifer microorganisms under denitrifying conditions,

whereas benzene was recalcitrant. However, Trizinsky and Bouwer (1990) observed biodegradation of benzene, toluene, and *m*-xylene in batch enrichment cultures, although *o*-xylene removal did not begin until the previous substrates were depleted. In contrast, other researchers have observed cometabolic biotransformation of *o*-xylene (Evans et al., 1991; Jørgensen and Aamand, 1991). Hutchins (1993) conducted microcosm tests with nonacclimated and acclimated aquifer material from Traverse City, MI, to assess the extent of biodegradation of radiolabeled BTEX as single substrates. The rates and extent of biodegradation of toluene and *m*-xylene in the acclimated aquifer material were generally similar to those observed in the nonacclimated material. Benzene was recalcitrant in both cases. *o*-Xylene was recalcitrant in the nonacclimated aquifer material, but degradation occurred after toluene addition. In the acclimated aquifer material, *o*-xylene degradation commenced without addition of toluene. Mineralization accounted for 36 to 54 percent of the total alkylbenzene removal. Thus, in general, these laboratory studies show that alkylbenzenes are degraded whereas benzene is recalcitrant when nitrate is used as the sole electron acceptor. However, these processes are not well understood at field-scale, where several other processes, including aerobic biodegradation, can proceed concomitantly.

There have been several field studies on nitrate-based bioremediation of fuel-contaminated aquifers. Results include: complete removal of benzene and toluene with the xylenes being more recalcitrant (Batterman, 1986); a 95 to 98 percent reduction in purgeable alkylbenzenes (Sheehan et al., 1988); complete removal of toluene with benzene, ethylbenzene, and the xylenes being unaffected (Lemon et al., 1989); and reductions of 87 percent , 67 percent, and 34 percent for toluene, ethylbenzene, and xylenes, respectively, with benzene being recalcitrant (Hilton et al., 1992). Other field tests are in progress (Hutchins and Wilson, 1994). However, these studies focus on aqueous concentrations and do not address whether BTEX levels are significantly reduced in the aquifer solids. Hutchins et al. (1991b) investigated the use of nitrate to promote biological removal of fuel aromatic hydrocarbons for a JP-4 jet fuel spill at Traverse City, MI, through a field demonstration project in cooperation with the U.S. Coast Guard. Although benzene was not degraded, laboratory tests had indicated denitrification would be a suitable alternative for biorestoration of the aquifer, (Hutchins et al., 1991b). The field work showed BTEX was degraded under denitrifying conditions in conjunction with low oxygen (microaerophilic) levels. However, a suitable control site was not available to test the effects of treatment without nitrate addition. Therefore, the relative contribution of nitrate to BTEX biodegradation in the field study requires further clarification.

To investigate this further, Hutchins et al. (1992) conducted two column tests using aquifer material to simulate the nitrate field demonstration project carried out earlier at Traverse City, MI. The objectives were to better define the effect nitrate addition had on the biodegradation of BTEX in the field study, and to determine whether BTEX removal can be enhanced by supplying a limited amount of oxygen as a supplemental electron acceptor. Columns were operated using limited (0.5-1.5 mg/L) oxygen, limited oxygen plus nitrate, and nitrate alone. In the first column study, benzene was generally recalcitrant in all three treatments, compared to the alkylbenzenes, although some BTEX removal did occur. In the second column study, nitrate was deleted from the feed to the column originally receiving nitrate alone and added to the feed of the column originally receiving limited oxygen alone. Benzene breakthrough was similar for each column.

Breakthrough of alkylbenzenes decreased by an order of magnitude once nitrate was added to the microaerophilic column, whereas alkylbenzene breakthrough increased by 50-fold once nitrate was removed from the denitrifying column. Although the requirement for nitrate for optimum alkylbenzene removal was clearly demonstrated in these columns, there were significant contributions by biotic and abiotic processes other than denitrification which could not be quantified.

2. The Need for Reproductive Toxicity Testing.

All animals proceed through a life cycle consisting of haploid and diploid generations. Cell and molecular processes may be very different at various points in the life cycle. This may cause a differential sensitivity to toxicants as some life stages may be especially sensitive because of specific cell receptors or processes. In terms of species survival, it matters little whether the cycle is interrupted at fertilization or during adult stages. Consequently, it is most important that toxicity screening assays account for different stages of the life cycle, especially the most sensitive ones such as reproduction and development. With small invertebrates that have a short life span, full life-cycle toxicity tests can be developed. However, when assessing vertebrate toxicity, it may be wiser to consider a battery of short-term tests that cover critical points of the life cycle. It also makes economic sense to use the same species for as many of these tests as possible. Therefore, the reproductive toxicity tests described here complement the more developed FETAX developmental toxicity test and a new neurotoxicity test presently being developed using *Xenopus* (J.T. Blankemeyer and J.A. Bantle-Research in Progress). *Xenopus laevis* (South African clawed frogs) are highly developed vertebrates that share many morphological and physiological similarities with mammals. They are plentiful, hardy, and fecund. Their fecundity allows a large number of gametes and offspring for statistical analysis. The experiments designed below test the effect of environmental contaminants on gametogenesis, reproductive behavior, and fertilization. Additionally, developmental effects caused by contaminants accumulated during oogenesis can be assessed.

3. Reproductive Toxicity Assay Design.

Gametogenesis and fertilization are highly specialized processes. In designing a reproductive toxicity test, it is critical that all of the important and/or specialized cellular processes involved are taken into account in the test design. Meiosis is the reduction of chromosomes from a diploid to haploid number and takes place only during gametogenesis. Interruption of meiosis can result in anueploidy or polyploidy as well as other chromosomal defects of a hazardous nature. In spermatogenesis, spermatogonial cells quickly undergo meiosis to become spermatids. However, in oogenesis in the frog, the primary oocyte is arrested in meiotic prophase until acted upon by gonadotropic hormones. It then proceeds through meiosis and arrests again in meiotic metaphase until fertilized by the sperm. It then completes meiosis, whereupon fusion of the pronuclei can occur. Meiosis is not the only important process as both sperm and egg undergo considerable morphogenic changes prior to acquiring their cell specific shape and function. In spermatogenesis, spermiogenesis is the subprocess whereby the spermatids slough unneeded cytoplasm and acquire their characteristic shape. The ovum also acquires yolk and develops specialized membranes and other structures. Finally, sperm and egg

must interact with one another in the process of fertilization in order to create a diploid embryo that can develop into a new individual.

The endpoints and exposures described above allow for continuous exposure during all of the previously mentioned cellular processes. For sperm and egg, their production, morphology, movement, and ability to fertilize were examined during the design of the reproductive toxicity assay. For meiosis, the ability to sustain subsequent embryonic development was monitored, although residual toxicants in the egg may have affected development as well. Missing genetic information or the effect of the toxicant on early development can be translated into malformed, stunted, or dead embryos. The assays described in this report are similar to many mammalian reproductive toxicity assays in design (Sullivan, 1988). These assays allow for a better comparison between the amphibian model and mammalian systems. One set of reproductive effects which is difficult to test by the proposed design are the effects of contaminants on the development of the gonad. It is possible that reduced production of Sertoli cells in the males or follicular cells in females during the early stages of life may affect fertility later. The number of primordial germ cells that find their way to the presumptive gonad during embryogenesis may also be affected by the toxicants as some xenobiotics function as environmental estrogens.

4. The Need for Developmental Toxicity Testing.

Developmental toxicity tests are designed to detect xenobiotic agents which affect embryonic development. Embryonic development can be considered a "weak link" in the life cycle of an organism. During this period, unique cellular and molecular processes operate to generate a complex multicellular organism from a zygote. These processes are sensitive and easily perturbed by many chemicals. Developmental toxicants are chemicals which can exert their effects at concentrations lower than that required to affect adults or cause general cellular toxicity. For example, semicarbazide causes malformation in frog embryos at 1/3000th the concentration required to kill embryos and affects embryonic growth at even lower concentrations (Schultz et al., 1988). Chronic full life cycle tests account for all significant life stages and usually take longer to run for vertebrates than the four day developmental toxicity test presented here. Short-term developmental toxicity tests can then be considered sub-chronic tests which may predict chronic effects in far less time and cost.

5. Development of FETAX as a Developmental Toxicity Screening Assay

The original work on developing a developmental toxicity screening assay using *Xenopus* embryos to detect environmental teratogens was performed in the laboratories of Greenhouse (1978) and Dumont et al. (1983a, b). Greenhouse used 48-hour exposures to military compounds (N-phenyl-β-naphthylamine and various hydrazines) to demonstrate toxic and teratogenic effects on developing embryos. Early studies in our laboratory have demonstrated FETAX can be used with a variety of chemicals and complex mixtures. The endpoints included: LC50 (mortality), EC50 (malformation-teratogenesis), no observed effect concentration (NOEC), minimum concentration to inhibit growth (MCIG) [both length and developmental stage], motor behavior, pigmentation, and gross anatomy. A Teratogenic Index (TI), the ratio of the 96-hour LC50/96-hour EC50 (malformation), was developed and has successfully been used as a measure of the

relative developmental hazard of a substance (Courchesne and Bantle, 1985; Sabourin and Faulk. 1987; Fort et al., 1988; Dawson et al., 1988; Dawson et al., 1989; Fort et al., 1989; Fort and Bantle, 1990a, b; Bantle et al., 1991a; Fort et al., 1991; Fort et al., 1992). Thus, assessment of teratogenic potential has been based on TI values, embryo growth, and types and severity of induced malformations. Generally, TI values less than 1.5 have indicated low teratogenic potential, whereas higher values have signified an increase in the potential teratogenic hazard. In these instances there was little or no separation between the concentrations which caused embryo malformation and concentrations which were embryolethal. Greater TI values signified a larger separation between the two responses, and thus, a greater possibility of embryos being malformed in the absence of significant embryo lethality. Types and severity of induced malformations have also been considered, especially for compounds with TI values less than 1.5 which produce serious defects of major organ systems. Such compounds may still pose a serious threat, possibly as embryotoxins.

We are establishing a database to assess the utility of the TI value in assessing teratogenic hazard. Test chemical exposures were continuous for 96-hours. Mortality and stage of development were checked at hours 24, 48, 72, and 96, while the other endpoints were recorded only at hour 96. Test compound renewal was performed daily throughout the tests. Data collection was simple, as all observations were made with a dissection microscope. The data collected using FETAX were in harmony with the criteria for an *in vitro* teratogenesis screen suggested by Kimmel et al. (1982). These criteria included: good concentration-response relationships, adequate number of embryos, and easily defined and quantified endpoints.

In addition, an American Society of Testing and Materials (ASTM) New Standard Guide for the Conduct of FETAX has recently been published (Bantle et al., 1991a). An "Atlas of Abnormalities" companion document to the ASTM guide has been produced in order to aid in the proper scoring of malformations (Bantle et al., 1991b).

6. FETAX Test Performance

Dumont (unpublished) has accumulated validation data on 45 compounds with nearly 85 percent correspondence to mammalian results. Sabourin and Faulk (1987) have completed testing of 32 compounds with 83 percent predictive accuracy. However, neither of these two investigators used the exogenous MAS developed for FETAX. MAS consists of Aroclor 1254-induced rat liver microsomes and a Reduced Nicotinamide Adenine Dinucleotide (NADPH) generator system. With over 100 compounds tested, we have approached 95 percent predictive accuracy in our laboratory using the *in vitro* MAS. Correlation between laboratories has been encouraging, as well. For example, Courchesne and Bantle (1985) found a TI for hydroxyurea of 4.3, whereas Sabourin recorded 4.5 for the same chemical. Interlaboratory validation study with several laboratories across the United States is currently being performed. Results obtained to date (Phases I (Bantle et al., 1994a) and II (Bantle et al., 1994b) eight test compounds) have been extremely encouraging and warrant further study. Phase III-Part I of this study has been performed in the blind, conducted by an independent technician weighing, bottling, and coding all test chemicals. These chemicals were then shipped to participating laboratories for independent testing. Phase III-Part I results were similar to those reported for Phase I (Bantle, et

al., 1996). Phase III-Part 2 (in press) tested each compound with and without the exogenous MAS. These results were excellent and similar to Phase II in that intralaboratory and interlaboratory variability were extremely low (unpublished).

Sabourin and Carlton (unpublished) determined the same stock of diphenylhydantoin caused pericardial edema as the primary anomaly in both the cultured whole rat embryo and *Xenopus* embryos. Dumont et al. (1983a) similarly found meclizine induced hydrocephalia in both frogs and mammals and other teratogens produced similar abnormalities in both frogs and mammals (developmental mimicry). Courchesne and Bantle (1985) reported a number of genotoxic chemicals caused the same general types of malformations in both *Xenopus* and rodent embryos. Dawson et al. (1989) have developed an artificial medium (FETAX solution) and have carried out a preliminary validation using five compounds ranging from a nonteratogen to a strong teratogen producing results corresponding to current mammalian literature. Fort et al. (1988, 1989, 1990a, b, 1991, 1992) have found 18 known mammalian teratogens tested with FETAX produced similar malformations in mammals.

C. SCOPE

1. The *In Situ* Bioremediation of Fuel Hydrocarbons

Research has shown clearly monoaromatic hydrocarbons, with the possible exception of benzene, can be degraded and, in many cases, mineralized under denitrifying conditions. In addition, other studies have shown fuel constituents, such as polycyclic aromatic hydrocarbons can be degraded under denitrifying conditions (Mihelcic and Luthy, 1988; Al-Bashir et al., 1990; Bouwer et al., 1992). The same holds true for aerobic breakdown products of fuel hydrocarbons, such as phenols, alcohols, and aromatic acids (Hu and Shieh, 1987; Dangel et al., 1989; Kuhn et al., 1988; Häggblom et al., 1990; Kluge et al., 1990; Rudolphi et al., 1991; Seyfried et al., 1991; Flyvbjerg et al., 1993). However, these types of compounds will in general be much more readily degraded under aerobic versus denitrifying conditions. Yet, given the problems inherent in promoting aerobic biodegradation of fuel hydrocarbons in anaerobic aquifers, there are significant advantages to using nitrate to supplement rather than replace oxygen for *in situ* bioremediation. Although denitrification has been considered to be an anaerobic process, it is not completely repressed in aerobic soil systems, and, in fact, low oxygen levels can even promote denitrification (Ottow and Fabig, 1985; Lloyd et al., 1987; Britton, 1989; Patureau et al., 1994). From a practical standpoint, several processes can be expected to occur under nitrate-based bioremediation because of the heterogeneity of aquifers and the establishment of microenvironments. In field tests to date, this has complicated the interpretation of the relative benefit of providing nitrate for *in situ* bioremediation.

2. Use of FETAX to Assess the Success of Bioremediation

As mentioned earlier, the proposed work is an extension of the previously developed FETAX test for developmental toxicants. The reproductive toxicity tests developed under this project use many of the same techniques and employ the same species as FETAX. The reproductive toxicity test complements the battery of short term tests now being developed by

the U.S. Army Biomedical Research and Developmental Laboratory to judge the potential hazard of environmental contaminants. Together, these tests examine reproductive, developmental, immune, neural, cancer, genetic and acute effects. The integrated test battery approach using lower vertebrates represents a cost-effective and relevant approach to human hazard identification. For the present project, the reproductive toxicity tests developed here have been helpful in evaluating the success of the nitrate-based bioremediation of the Eglin AFB site.

3. Summary of Research Objectives

The objective of this research was to compare the extent of bioremediation using aerobic recharge with and without nitrate addition. Our intent was to evaluate the benefit of providing nitrate as a supplemental electron acceptor under field conditions. In addition, this project provided an opportunity to evaluate whether nitrate-based bioremediation would have any effect on native microbial populations. FETAX reproductive and developmental toxicity tests would provide an indication as to whether remediation was increasing or decreasing toxicity. If toxicity was eliminated, then the remediation process could be terminated before all contaminants were removed from the soil.

SECTION II
SITE CHARACTERIZATION

A. SITE DESCRIPTION

Extensive site characterizations by other groups have been published elsewhere and are available (Weston, 1984; EA Engineering, 1987; EA Engineering, 1993). In brief, the field site is located within the Petroleum, Oils, and Lubricants (POL) facility at Eglin AFB, FL (Figure 1). The terrain is relatively flat, with the subsurface consisting of a 30-40 ft thick shallow sand-and-gravel aquifer which extends down to contact the Pensacola Clay confining unit. The aquifer dips to the south-southwest at a rate of 15-25 ft per mile. The estimated porosity is 35 to 45 percent, and the horizontal and vertical conductivity are approximately 0.5 ft/day (Weston, 1984).

In April 1984, a leak in an underground jet fuel pipeline was detected by Air Force personnel (Figure 1). A preliminary site characterization estimated that 30,000-40,000 gallons of JP-4 jet fuel had contaminated approximately 4,000 cubic yards of soil and shallow aquifer material. Use of the pipeline was discontinued, and a series of shallow, gravel-filled trenches were installed perpendicular to the direction of fuel movement. By October 1984, skimmer pumps had recovered 7,400 gallons of JP-4. By 1986, free product had been reduced to levels which were nonrecoverable, and the use of the skimmer pumps was discontinued.

In 1986, EA Engineering conducted additional site characterization to prepare for installation and operation of a pilot demonstration project of enhanced *in situ* biodegradation using hydrogen peroxide (EA Engineering, 1987). A system was designed for delivering nutrients and hydrogen peroxide to the subsurface via three application methods: 1) injection wells, 2) infiltration galleries, and 3) spray infiltration (Figure 2). Four recovery wells were installed to provide ground water for recirculation. The application system was constructed and put into operation in March 1987. Over an 18-month period, approximately 7,800 pounds of inorganic nutrients and 94,000 pounds of 35 percent hydrogen peroxide were injected into the subsurface. Problems with both hydrogen peroxide stability and loss of infiltration capacity were encountered, which reduced delivery of oxygen to the subsurface (Hinchee et al., 1989). Approximately 5,000 pounds of JP-4 hydrocarbons were removed, with volatilization accounting for approximately 70 percent of the total removal.

B. NRMRL/RICE SITE CHARACTERIZATION

The proposed treatment area for the current study on nitrate-based bioremediation encompasses the area affected by the hydrogen peroxide study (Figure 2). Operation of the hydrogen peroxide delivery systems undoubtedly had significant effects on the subsurface hydrology, microbiology, and contaminant distribution. In addition, there had been no site characterization for 5 years following the hydrogen peroxide study. Finally, specific parameters required for thorough evaluation of nitrate-based bioremediation were not obtained during previous investigations. Therefore, additional site characterization was required to provide information for design and operation of the nitrate-based pilot demonstration system.

Personnel from the NRMRL, Rice University (Rice), and Oklahoma State University (OSU) coordinated and conducted several field trips to Eglin AFB during 1993-1994. The objectives were to: 1) define stratigraphy and hydraulic conductivity using cone penetrometry, 2) provide water quality information with respect to both sample depth and aerial coverage, 3) obtain continuous core samples through the contaminated interval at several locations across the site to delineate fuel mass and distribution, 4) obtain both water and core samples for column studies to assess plugging potential, and 5) conduct a combined infiltration/tracer test in each proposed treatment cell to evaluate the depth of penetration of the recharge water and develop hydraulic parameters for modeling purposes.

1. Cone Penetrometer Survey

In March 1993, researchers from NRMRL and Rice conducted a comprehensive site investigation at the POL facility to characterize site hydrogeology, determine the spread and vertical extent of BTEX and JP-4 contamination in aquifer core samples, and provide vertical resolution of water quality. This field activity involved the use of a cone penetrometer, geoprobe, and conventional drilling rigs. A cone penetrometer operated by Terra Technologies, Inc., was used to assess areas of BTEX contamination and associated dissolved oxygen as well as to characterize the hydrogeologic properties at the subsurface at the site. Sampling points were installed at the water table in 26 locations to measure BTEX and dissolved oxygen concentrations across the site (Figure 3). Collected samples were analyzed for BTEX on a real-time basis using a portable GC. This methodology allowed a rapid assessment of the contaminant plume, since collected data could be analyzed and used to delineate additional sampling points. For quality control, 17 split samples were preserved and shipped to NRMRL for Gas Chromatography/Mass Selective Detector (GC/MSD) analysis. With the exception of two anomolous readings (CPT-8, CPT-9), laboratory and field analytical results agreed quite well ($r^2 = 0.9986$). The anomolous data were not used. A maximum BTEX level of 4,500 mg/L was detected, with levels decreasing to approximately 10 mg/L over a distance of 300 ft downgradient of the spill (Figure 4). Lateral spreading of the plume was identified over a distance of 350 ft. Dissolved oxygen (DO) levels measured in the field were consistently below 1 mg/L across the investigated area.

The detailed stratigraphy provided by the cone penetrometer typically identified sand from the ground surface to the depth of penetration (15 to 20 ft). Clay lenses were detected at about 15 ft in several locations. One cone hole (CPT-14) was completed to a depth of 33 ft where a clay aquitard identified by previous investigators was encountered. Water table elevations determined by the cone penetrometer provided data for a potentiometric map, indicating the ground water flow generally follows land surface contours as shown in Figure 1. Interpretation of the cone logs suggest the conductivity of the sand ranges from 0.010 to 0.045 cm/s.

2. Water Quality Analyses

a. Methods

Several parameters were monitored to provide an extensive characterization of water quality and indicate the types of microbial processes which may have been occuring in the subsurface. Because the water table was very shallow, samples were collected using either peristaltic pumps or submersible pumps. Flow-through systems were used to minimize contact with air so samples could be analyzed in the field for DO and pH using electrodes. In addition, samples were analyzed immediately for soluble iron using a Chemetrics® photometric method. Duplicate samples were taken for BTEX and TOC by filling 40-ml VOA bottles and acidifying to pH less than 2 with H_2SO_4. These were sealed without headspace using Teflon-lined septa. Duplicate samples were also taken for dissolved gases by overfilling 60-mL glass serum bottles, acidifying to pH less than 2 with H_2SO_4, and crimp-sealing without headspace using Teflon-lined grey butyl rubber septa. Samples for nutrients and inorganic parameters were collected in clean plastic containers. All samples were refrigerated and/or stored on ice for transport to NRMRL.

To evaluate volatile aromatic hydrocarbons, samples were analyzed for trimethylbenzenes as well as BTEX. The trimethylbenzenes include mesitylene (MESIT), pseudocumene (PSCU), and 1,2,3-trimethylbenzene (TMB). Taken collectively, this combination will be referred to as BTEXTMB. Samples were analyzed using a Varian Saturn II Mass Spectrometer in combination with a Varian 3400 gas chromatograph and a Tekmar 7000 Headspace Autoanalyzer.

Dissolved gases were analyzed by replacing part of the water volume in the sealed serum bottles with helium, and then sampling the equilibrated headspace (Kampbell et al., 1989). Methane, carbon dioxide, and nitrogen were analyzed using a Hewlett-Packard 5890 gas chromatograph with a thermal conductivity detector. Bromide, chloride, and sulfate were analyzed using a Quantum 4000 (Waters) capillary electrophoresis unit.

b. Monitoring Wells

There are several wells located at the POL area which had been installed over the past 10 years. However, well logs and construction records could not be found for some of these. In addition, most of the existing wells are screened over large intervals, providing little information on water quality in localized zones of contamination. Because of this, many of the wells at the site were not used in this study. Also, additional wells were constructed during site characterization as part of this and other ongoing investigations. Those wells shown in Figure 1 were periodically sampled to provide background information and to assess the effects of pilot operation outside of the treatment cells. Details of well construction are shown in Table 1. Water quality analyses for the monitoring wells at different time periods are shown in Table 2. Because EPA Wells 1-4, 5B, 5C, 83-1, 83-2, and 83-7 were installed after the initial sampling trip, background water quality data are not available for these locations. The data indicate the general anaerobic nature of the aquifer, with pH values generally less than 6.5, DO values less than 1.0 mg/L, and methane concentrations up to 15 mg/L. The lower zones of the aquifer, sampled by the PL wells, appear to be somewhat less anaerobic, with lower methane concentrations, higher

sulfate levels, and less contamination. However, significant concentrations of BTEXTMB were present throughout the aquifer, especially in the vicinity of the original treatment area (Table 2, Figure 1). Benzene concentrations were reduced relative to the other constituents, probably as a result of weathering and the pilot project on hydrogen peroxide treatment. However, concentrations exceeded compliance levels in several locations. Very little nitrate was originally present, but nutrients such as ammonia-nitrogen and phosphate were relatively high, especially in the original treatment area. These data show the overall aquifer was still contaminated, and the subsurface may be conducive to nitrate-based bioremediation.

c. Geoprobe Samples

Although the data provided by the monitoring wells gave a general picture of the state of the aquifer, there was insufficient vertical resolution to ascertain the water quality status in the proposed treatment area. NRMRL researchers therefore used a Geoprobe to drive a screened rod to three selected depths at several locations to obtain water samples for correlating water quality information with core analyses. Locations of the Geoprobe sample points are shown in Figure 5, and the water quality data are shown in Table 3. Again, DO values were low, especially from 7 to 11 ft below ground surface. Ammonia-nitrogen concentrations tended to increase with depth at most locations. One explanation for this is that nitrification of applied fertilizer produces nitrate in the rhizosphere, which is then reduced to ammonia through dissimilatory nitrate reduction as the nitrate infiltrates through the contaminated region. This would happen with an aerobic soil zone and an anaerobic subsurface, providing there was sufficient available carbon. This would appear to be the case in the treatment area, since both TOC and BTEXTMB levels were high throughout the aquifer. In addition, sulfate levels were low and methane levels were high, with higher methane concentrations generally within the deeper regions of the aquifer. This would tend to indicate the aquifer microorganisms are metabolically active in this anaerobic environment. Benzene concentrations ranged from 0 to 300 mg/L and were erratically distributed with respect to total BTEXTMB (Table 3). This could indicate selective volatilization, leaching, or biodegradation, depending on the depth of the water sample and proximity to the original spill area. However, it could also indicate the presence of other spills. For example, the ratio of benzene to total BTEXTMB was 3 percent nearest the spill location (80E-2), 13 percent downgradient of the spill (80I-2), and 0.4 percent in the far corner of the proposed control cell (80H-3). However, the corresponding BTEXTMB levels were 2550, 2280, and 24100 mg/L in those locations. This does not correlate with preferential leaching of benzene from the original fuel spill. Without data from these locations prior to the fuel spill, it is difficult to determine whether all of the contamination at the site originated from the JP-4 jet fuel pipeline leak. Nonetheless, these data show that despite the aerobic bioremediation provided by the hydrogen peroxide demonstration project, extensive contamination of the ground water occurs over the project area to a depth of at least 11 ft below ground surface.

3. Core Analyses

Core samples were taken on several separate occasions for various purposes. This section describes sampling, analytical methods, and results for the measurement of BTEXTMB and JP-4 in aquifer cores. This was done to delineate the lateral spread and vertical extent of contamination at the site and provide mass estimates. This information was also used to help define the locations of the proposed treatment cells.

a. Methods

Core samples were obtained using a Giddings probe modified for acquistion and extrusion of saturated aquifer material. Samples were collected using 2-in hollow core barrels either with or without pistons to prevent loss of flowing sands (Leach et al., 1989). Cores were extruded into sterile, clean half-pint Mason jars using a paring device to shave off the core material which had been in contact with the core barrel. The jars were immediately sealed and set aside until the entire core barrel had been emptied. Each core was then subsampled using a sterile, clean 10-mL tuberculin syringes with the tip removed. The core was subsampled to the bottom of the jar to provide a subsample representitive of the entire core length. The subsample was immediately added to a tared 40-mL VOA vial containing 5 mL deionized water and 5 mL methylene chloride, and the vial was sealed with a teflon-lined silica septum and mixed. Extract vials were either stored on ice or at at room temperature prior to transport to NRMRL for analysis.

Sample vials were weighed to determine mass of core sample added, and samples were then extracted by placing on a wrist-action shaker for 30 min and sonicating for 1 min. The organic extract was removed with a syringe, passed through a sodium sulfate column, and fire-sealed in a glass ampule. For JP-4 analyses, samples were analyzed using a Hewlett-Packard 5880 GC with a flame ionization detector. Samples were chromatographed on a 30-m x 0.53-mm DB-5 capillary column with 1.5-mm film thickness. The column was temperature programmed from 10°C (3.0 min) to 56°C at 4°C/min, then to 75°C at 30°C/min, then to 95°C at 2°C/min, held for 1 min, and then to 254°C at 30°C/min with a final 8.0-min hold. The column flow rate was 4.7 mL/min. JP-4 concentrations were quantified with a 7-point external standard calibration curve ranging from 50 to 50000 mg/L. The detection limit was based on the initial mass of core sample; with core samples averaging around 30 g, the detection limit was approximately 10 mg/kg on a wet weight basis.

BTEXTMB was quantified using a Hewlett-Packard 5890 GC equipped with a Hewlett-Packard 5971 MSD. Cool (38°C) on-column injection was used with electronic pressure control set for a constant flow of 0.9 ml/min. A 30-m x 0.25-mm Restek Stabilwax capillary column with 0.5-mm was used, preceded by a 230-mm x 0.53-mm uncoated capillary precolumn. The column was temperature programmed from 32°C (3.0 min) to 70°C at 4°C/min, then to 200°C at 20°C/min with a final 1.0-min hold. Quantitation was based on calibration curves of a single target ion for each compound with the addition of up to two qualifier ions recorded to verify chromatographic separation or purity. The ions chosen were those listed in EPA Method 524.2 (Revision 3.0). Both low-level (0.01 to 10 mg/L) and high-level (10 to 300 mg/L) calibration curves were used, with fluorobenzene as the internal standard. The system detection limit was

0.02 mg/L, which provided for a method detection limit of approximately 0.003 mg/kg on a wet weight basis.

Selected core extracts were also subjected to an extensive GC/MS search to better define the distribution of the residual volatile hydrocarbons. Samples were chromatographed using a 30-m x 0.25-mm Restek Stabilwax capillary column with 0.5-mm film thickness coupled to a 100-m x 0.25-mm DB-1 Petrocol column with a 0.5-mm film thickness. Data were obtained in a scan mode (m/z = 34 to 450) and peak spectra were compared with library spectra to provide tentative identifications. These identifications were then sorted into separate compound classes using a computer program. A final manual spectral interpretation was made for all compounds which were not identified or where significant coelution was observed. A "calibration curve" was created from the analysis of 117 different petroleum compounds, including alkanes, alkenes, cycloalkanes, monoaromatic hydrocarbons, and polycyclic aromatic hydrocarbons. This curve was used to relate response factor to retention time ($r^2 = 0.977$), and provided a semiquantitative analysis of the weight percent of the various compound classes. For comparative purposes, concentrations of individual monoaromatic hydrocarbons (BTEXTMB) were also done this way.

b. Results

Initially, 20 locations were designated for the acquisition of continuous cores, including two which extended from ground surface to 20 ft below grade. The locations of these cores are shown in Figure 6. Core locations 80A through 80J also correspond to the locations used for taking geoprobe samples, thus providing a direct comparison between core samples and water quality analyses. For each core location, concentrations of BTEXTMB and JP-4 in the individual subsamples were weighted for the sampled interval and summed to provide a total cumulative mass estimate in g/m^2 for that location. A bulk density of 1830 kg/m^3 was assumed. Cumulative mass data for all of the core samples are shown in Table 4. Based on these analyses, Rice personnel provided a contour plot showing the cumulative mass of JP-4 (in g/m^2) across the site (Figure 7). The source was located in the proximity of 80N-80S, adjacent to the fuel tank, and the resultant residual saturation was found distributed fairly evenly across an area downgradient. The contaminated interval was 4 to 5 ft thick adjacent to the source, but was generally 2 to 3 ft thick downgradient. The bottom of the contaminated zone (<20 mg/kg JP-4) ranged from 4 to 7 ft below land surface. Based on a 300-ft x 300-ft area which encompasses all 20 core locations, the total JP-4 mass was estimated to be 26800 kg (T. Fisher, personal communication). This is equivalent to 9300 gallons, assuming a density of 0.76 (Smith et al., 1981). In the 100-ft x 200-ft proposed treatment area, the JP-4 mass was estimated to be 2860 kg, based on the analyses of core locations strictly within the treatment boundaries. At the time of the initial sampling (March, 1993), most of the JP-4 was located below the water table in the majority of the locations for which water table information was available.

Subsamples were taken from each of the core locations, generally representing the most contaminated interval, and analyzed for distribution of compound classes relative to JP-4 fresh fuel samples (Table 5). In general, weathering has reduced the aromatic and cycloalkane fractions by 3 percent and 4 percent, respectively. Core locations 80D and 80I are unusual in that the alkane fractions are significantly higher than those in the other cores. For location 80I, the high

benzene concentrations in the soil and water, coupled with the extent of surface soil contamination, suggests that this may have resulted from another source, perhaps spillage from the surface transfer station. The last four cores in Table 5 had very low "JP-4" levels, and therefore the distribution of compound classes may not be valid. However, core analyses revealed that there may be deeper plumes which probably originate from another upgradient location. This is shown by high levels of benzene and toluene, but not alkanes, in the soil 9 ft below surface at location 80H, and is substantiated by the Geoprobe water quality information from that location as well. For example, the weighted average core concentration of toluene in cores 80H8 through 80H11, covering the depth interval 7.2 to 8.7 ft below ground surface, is 0.208 mg/kg. Assuming a bulk density of 1830 kg/m^3 and a porosity of 30 percent, the expected aqueous concentration of toluene, excluding sorption, would be 1270 mg/L. The Geoprobe location 80H-2, screened from 7.2 to 8.7 ft below ground surface, yielded water with a toluene concentration of 940 mg/L.

Analysis of the JP-4 jet fuel reveals that BTEXTMB makes up about 45 percent of the total aromatics, and the total aromatics make up about 17 percent of the JP-4. In contrast, based on analysis of BTEXTMB concentrations in the core samples listed in Table 5, BTEXTMB makes up about 2-36 percent of the total aromatics in the weathered cores, with the higher percentages closer to the spill area. The total aromatics make up about 14 percent of the residual JP-4. These weight percentages can be used to estimate total nitrate demand. If it is assumed the treatment area contains 2860 kg of JP-4, 14 percent of which are aromatics, this yields 400 kg of aromatics. A conservative estimate would be that 20 percent of the aromatics can be degraded under denitrifying conditions, leading to a nitrate demand of 80 kg NO_3-N for both treatment cells, assuming complete denitrification (Hutchins et al., 1991b). Actually, other sinks for nitrate will probably lead to increased nitrate consumption beyond that afforded by the labile aromatic hydrocarbons.

D. TIME COURSE AND SAMPLING INTERVAL OF NITRATE-BASED BIOREMEDIATION

The initial sampling at the Eglin AFB site was conducted March 22-25, 1993. Toxicity tests conducted during this time period were termed pre remediation testing. Square test and control cells were then marked off, sprinkler systems installed and nitrate treatment (NO_3-N) commenced on April 7, 1994 at a rate of 10 mg/L. The control cell was treated with water only throughout the study. Nitrate levels were increased to 15 to 20 mg/L on July 15, 1994. On August 19-30, 1994, interim core sampling was performed and toxicity tests were performed which were termed during remediation testing. Because lysimeter data from August sampling showed incomplete transfer of nitrate, plots were stripped and weed barriers installed November 14 to 16, 1994. The final performance evaluation was conducted May 13 to 30, 1995 and termed post remediation toxicity testing.

SECTION III

FETAX TOXICITY TESTING

A. PRELIMINARY TESTING

1. JP-4 Exposure Testing Using Agarose

a. Procedure.

FETAX was employed to initially evaluate the toxicity of JP-4. A modified protocol was used which permitted the testing of hydrophobic materials such as JP-4 with the FETAX assay. Modifications included using a 2 percent solution of electrophoresis grade agarose along with different concentrations of JP-4 suspended in the agarose. The agarose was prepared normally, then aliquoted into 7 mL portions. These portions were then mixed with the appropriate concentration of JP-4 at a temperature immediately above jelling. This solution was then vortexed to fully integrate the JP-4 into the agarose. The mixture was then allowed to solidify on the bottom of Petri dishes. After the agarose-JP-4 mixture was solidified, 8 mL of FETAX salt solution was added to each Petri dish along with 20 embryos. This method allowed the JP-4 to slowly leach out and expose the embryos on top on the agarose. Although the FETAX solution was not tested for JP-4 components, the micells in the agarose became smaller through time and the embryos responded to the exposure. Every 24 hours, the 8 mL of FETAX solution was removed and replaced with fresh FETAX solution and dead embryos were removed. The test was stopped after 96 hours and mortality and malformation data were collected following standard FETAX procedures.

b. Preliminary tests.

The first preliminary test (JP-4#1--See Table 6 for a key to test abbreviation names) had JP-4 concentrations which ranged from 0.125 to 12.5 percent. Five different concentrations of JP-4 were prepared: 0.125 percent, 1.25 percent, 3.125 percent, 6.25 percent, and 12.5 percent. The concentrations and the amount of JP-4 and FETAX used to make each concentration are shown in Table 7.

Test 1 results showed that JP-4 was developmentally toxic to embryos. The 12.5 percent JP-4 concentration killed nearly all of the embryos after 96 hours. Mortality data is shown in Table 8, and malformation data is shown in Table 9. Although the data were preliminary, the 96-hr LC50 was 8.4 percent and the EC50 (malformation) was 3.5 percent and the TI was 2.4. TI values above 1.5 pose a potential developmental hazard. The initial conclusion was that JP-4 was developmentally toxic and that further experimentation was warranted based on these results. Results from Test 1 are detailed in Table 10. Although the percent malformation and percent mortality of the control dishes exceeded the maximum 10 percent mortality in ASTM guidelines for this test, it did show the JP-4 was an obvious cause of a concentration-response in FETAX.

The next pair of preliminary tests (JP-4#2 and JP-4#3) compared the use of the MAS to a test using the same clutch of eggs without MAS. MAS consists of a generator system and Aroclor 1254 rat liver microsomes co-cultured with *Xenopus* embryos performed to determine the potential human health hazard a contaminant may pose. When co-cultured with *Xenopus* embryos, MAS simulates the mammalian liver and placenta in activating proteratogens and deactivating others. Consistent use of MAS makes comparison to human developmental toxicity more predictively accurate.

The malformation data for these two tests are given in Tables 11 and 12. Mortality data for these two tests are given in Tables 13 and 14. Results from these tests are summarized in Tables 15 and 16. The LC50 with microsomes was 7.7 percent, and the EC50 with microsomes was 3.0 percent. The LC50 without microsomes was 7.7 percent, and the EC 50 without microsomes was 2.3. The 96-hour LC50 was essentially unchanged, but the 96-hour EC50 (malformation) was increased, thus changing the TI from 3.3 to 2.5. It was, therefore, concluded that the developmental toxicity was somewhat reduced by the inclusion of microsomes, but there was no significant microsomal effect. These tests were considered preliminary, because neither passed ASTM guidelines for percent mortality and percent malformation in the control dishes.

The results from Tests numbered 4 and 5 (JP-4#4 and JP-4#5) were performed using the same procedure as the prior two tests. JP-4#4 was performed without microsomes, and JP-4#5 was performed with microsomes. Malformation data, mortality data, and results from JP-4#4 are given in Tables 17, 18, and 19. Malformation data, mortality data, and results from JP-4#5 are given in Tables 20, 21, and 22. The LC50 for JP-4#4 was 8.8 percent JP-4, and the EC50 (malformation) was 2.0 percent JP-4. The percent mortality for the control in JP-4#4 was 26 percent, and the percent malformation in the control was 10.2 percent. Neither of these values was less than the maximum allowable values as stated in the ASTM guidelines. The LC50 for JP-4#5 was 10.30 percent JP-4, and the EC50 (malformation) was 1.8 percent JP-4. The percent mortality for the control in JP-4#5 was 5.0 percent and the percent malformation in the control was 10.5 percent. The percent malformation value was slightly larger than the maximum allowable value as stated in the ASTM guidelines. However, this test was considered acceptable based on the low mortality rate.

Test JP-4#6 also assessed toxicity of JP-4 through a modified FETAX test without MAS. This test involved exposing the embryos to the JP-4 while it was suspended in agarose, as in the previous tests. Mortality and malformation data are given in Tables 23 and 24, and results are summarized in Table 25. The LC50 for test JP-4#6 was 9.4 percent JP-4, and the EC50 (malformation) was 2.3 percent JP-4. These values resulted in a TI of 4.2. This test was considered acceptable because both percent mortality and percent malformation values for the control dishes were below the maximum allowable values as stated by the ASTM guidelines.

Test JP-4#7 was performed with MAS. Malformation data, mortality data, and results summary are given in Tables 26, 27, and 28. The LC50 for test JP-4#7 was 12.3 percent JP-4, and the EC50 (malformation) was 1.9 percent JP-4. These values gave a TI of 6.47. The percent malformation of the control dishes was slightly greater than the maximum allowable value as stated by the ASTM guidelines. Since the mortality value was only 1.0 percent, this test is considered acceptable.

Table 29 is a summary of the LC50, EC50 (malformation) and TI for the tests involving direct JP-4 exposure performed by suspending the JP-4 in agarose. These data suggest that mortality was reduced slightly (detoxification) upon microsome addition. Malformation was not greatly changed. This led to a slight increase in TI after microsome addition. The TI for these last experiments was fairly high, suggesting some teratogenicity. The concentration-response curves for both mortality and malformation curves were nonlinear, making the interpretation of the TI more difficult. The ASTM guidelines for control mortality and malformation rates were developed for the standard aqueous FETAX assay and used here as a reference. Because test dishes (including controls) were lined with agarose, the percent malformation and percent mortality were expected to be higher with agarose probably due to soluble components in agarose.

2. Testing JP-4 and Weathered JP-4

To determine whether JP-4 toxicity was due to light, volatile components or the heavier elements, it was decided to artificially "weather" the JP-4 as Eglin AFB soil samples would be more like weathered JP-4. This experiment allowed a more direct comparison of toxicity.

a. Procedure.

Air was bubbled through 100 mL of JP-4 until only 80 mL remained. The volume that was evaporated was assumed to be the BTEX fraction although a chemical analysis was not performed. A FETAX test was then performed using the weathered JP-4 and regular JP-4 in a series of concentrations in agarose.

b. Preliminary Tests.

The JP-4 versus weathered JP-4 test (WJP-4#1) was conducted and there was almost no difference between JP-4 and weathered JP-4. The results are summarized in Table 30. The control mortality and malformation were 1.25 percent and 3.8 percent, respectively. 100 percent malformation was observed in all other concentrations. Mortality was low in all concentrations below 3.75 percent and was 100 percent in concentrations of 3.75 percent and greater. Control values for both percent malformation and percent mortality fell within ASTM guidelines. Experiments JP-4#1 to JP-4#7 showed similar results with LC50 values between 7.7 to 9.4 percent without MAS (Table 29). However, Table 29 shows that the LC50 is less than 3.75 percent for JP-4. The reason for this result is unclear, but since the same clutch of embryos was used for "weathered and unweathered" JP-4, the results still indicates that removal of lighter JP-4 components does not affect toxicity.

The JP-4 experiments showed that jet fuel is embryotoxic but not greatly teratogenic. Although there was an indication of toxicity attenuation by MAS, it was not great enough to warrant inclusion of MAS in future experiments. A two-fold increase or reduction in toxicity would necessitate the use of the MAS. This finding was not present in the test results. The agarose technique, while somewhat variable for one experiment, proved acceptable for use in the supercritical fluid extraction tests described below.

With the toxicity of JP-4 established, work commenced on how best to expose embryos to the Eglin AFB soil samples. Three methods were chosen for evaluation. These were SCFE, aqueous extraction and direct exposure techniques.

3. Background Testing for SCF FETAX Tests

a. Procedure.

After the preliminary JP-4 tests were complete, an evaluation of extraction and exposure methods was performed. The experiments presented here used JP-4 instead of SCF extract to determine whether toxicity could be observed. In these tests, JP-4 was mixed with corn oil in a 1:1 ratio, and the mixture was injected into an agarose base. It was anticipated that SCFE would have to be solubilized in a vehicle such as corn oil. Therefore, preliminary testing used corn oil to determine its toxicity and other properties. These tests also attempted to address and correct the concerns encountered in the first round of JP-4 and agarose tests which were performed. It was thought that some of the JP-4 was lost due to volatilization once it was placed in hot agarose. To solve this problem, low melting point agarose (<35°C) was purchased and used in subsequent testing. This new agarose was used in all succeeding agarose tests. Another concern was the variation of the size of the bubbles of the JP-4/corn oil in the agarose. We attempted to emulsify the JP-4/corn oil and the water before injecting it into the agarose, rather than injecting them separately. The size of the bubbles with the new method was more uniform, but in addition to the better uniformity, a greater amount of the JP-4/corn oil was held in the agarose base (Figure 8). This method was used in all following tests.

b. Preliminary tests.

The first test using JP-4, corn oil and agarose (JCA#1) tested the same JP-4 concentrations as the previous JP-4 and agarose tests, but the resulting LC50 and EC50 values were different. For FETAX Test JCA#1, the LC50 was 6.9 and the EC50 was not able to be calculated due to 100 percent malformation in the experiment. The malformation data and results summary are given in Tables 31 and 32. This test was considered preliminary because the control dish values for both the mortality and malformation were above the maximum allowable values set by the ASTM guidelines.

The results from the second test using JP-4, corn oil and agarose (JCA#2) were reported in Table 33. The malformation and mortality results in the control dishes of this test were greater than the ASTM maximum values. Therefore, LC50 and EC50 values were not calculated for this test.

Third and fourth tests were performed to compare the results with and without corn oil using the low-melting point agarose. In test JCA#3, JP-4 was mixed in a 1:1 ratio with corn oil (Tables 34-36), and in test JCA#4, JP-4 was used without corn oil (Tables 37-39). The concentrations were injected into the low melting temperature agarose. This resulted in lower LC50 values than in our previous tests, and no EC50 values due to 100 percent malformation at our lowest concentration. A comparison of the two tests revealed a slightly lower LC50 value in the JP-4/corn oil test (2.3 compared to 3.0 in the test without corn oil). This was somewhat expected, because more of the JP-4 remained in the exposure chamber after the introduction into the agarose. This greater concentration of JP-4 would be more toxic.

4. SCF FETAX tests

a. Procedure.

SCFE was performed at NRMRL. The FETAX portion of the SCF test was performed in a similar manner to the JP-4 and agarose experiments. Seven mL of low gelling temperature agarose were placed in a Petri dish, and the predetermined amount of SCF was then injected into the agarose. Eight mL of FETAX solution were then overlaid on the agarose and the agarose was allowed to cool. Twenty embryos were then placed in the FETAX solution.

b. Preliminary Tests.

The first preliminary test (SFC#1) was performed with the SCF from the 'B' site as there was more of the extract from that site than from any of the others. Sixty µl of the SCF was injected into the agarose, mixed vigorously and poured into a Petri dish. After gelling, FETAX solution was added and then 20 embryos were added. Sixty µL of extract was similar to the amount of TPH which theoretically would have been extracted from 15 grams of soil from site B, according to results of chemical tests performed. Fifteen grams of soil was chosen as an amount that should have an effect on the embryos. All the embryos in the extract were dead at 48 hours. Control mortality and malformation were both less than 15 percent, but above ASTM Guideline Maximums. Table 40 contains a comparison of the concentration of extract used versus a similar concentration of JP-4 used. Forty µL was the lowest concentration of JP-4 that was tested, and 300 µL was the lowest concentration which caused 100 percent mortality. The SCF was at least 5 times more toxic than the JP-4. Some possible reasons for this difference are: previous bioremediation efforts may have made the JP-4 more toxic; the JP-4 may have bound to other toxicants in the soil; or the SCFE process may have concentrated the more toxic elements of JP-4.

In SCF FETAX Test #2 (SCF#2), SCF samples were extracted from the B, G, O, and S sites (see Figure 32), and a FETAX test was performed with agarose. Results from this test are detailed in Table 41. The volumes used for each site were B-20 µL/dish/day, G-35 µL/dish/day, O-17 µL/dish/day, and S-43 µL/dish/day. These volumes were calculated to be equivalent to the volume of TPH in 15 grams of each soil type. The extraction efficiency was based on the TPH present, as determined by environmental chemistry versus actual recovery by weight of SCF. The weight of SCF was converted to volume of SCF, and the volume increased to account for loss during extraction. The experiment then attempted to expose embryos to the same amount of JP-4 as later direct exposure experiments which also used 15 grams of soil. There was not enough SCF to establish a concentration response curve so there was only one concentration used for each site. The dose of SCF was apparently too high, because only the G site had living embryos at the end of the exposure. This suggests that SCF was more toxic than the estimated JP-4 present in the soil sample.

5. Assessing the Toxicity of the SCFE Process

The purpose of the next experiment was to determine whether toxicity increased during the SCFE process. This experiment was prompted by the observation that the SCFE were highly toxic to the embryos. This experiment involved mixing corn oil with commercial grade blasting sand. A SCFE was performed on the sand and then the extract was mixed with agarose.

Embryos were then exposed to the SCFE-agarose mix in a standard FETAX test. This test (SAND#1) was set up following the concentrations of SCFE given in Table 42. Embryos were placed in each dish and incubated at 24°C overnight. Test dishes were examined for mortality the next day. There was high mortality in the control dishes as the blasting sand alone sometimes contributed to toxicity. However, there was no survival in any of the SCF exposure dishes. Death occurred promptly at gastrula. The test was terminated at 24 hours because of the total mortality in the SCF group. The two possible sources of toxicity were the blasting sand and the SFE apparatus. The same bag of blasting sand was used for all tests discussed in this report.

The next step was to assess the effects of blasting sand on *Xenopus* development. Embryos were exposed to the same blasting sand used in the SCF experiment described above. If there was no toxicity in blasting sand, then the toxicity would either be due to concentrations of low levels of toxicants in the blasting sand which were concentrated by supercritical fluid extraction or the apparatus itself. Test SAND#2 was performed to assess this. Commercial grade silica was also tested as a possible substitute for blasting sand in future experiments. Standard FETAX solution controls in Petri dishes were used to ensure good embryos and FETAX solution. This test was set up as a direct exposure experiment. Four sediment free jars with inserts were set up and filled with 140 mL of FETAX solution. Each jar in the first sediment exposure group contained 35 grams of silica. The second group of jars held 35 grams of blasting sand. 140 mL of FETAX solution was added to each jar after insertion of the glass tubing. Thirty embryos were placed on the stainless steel mesh of the inserts in each jar; see Figure 9 for a diagram of the insert and jar. Controls were performed in standard glass Petri dishes to assess embryo quality without the added variability that could be added by the jars. These Petri dishes contained 25 embryos. Testing jars as well as their contents were changed every day for the duration of the experiment by carefully removing the inserts from the jars and placing them in the newly prepared jars. The test was carried out for 96 hours. Oxygen content was slightly low, but the controls survived well. The pH values were acceptable. The mortality rate in the controls ranged from 4 percent to 5 percent which was within the expected values (Table 43). There was high mortality in one of the silica jars, but this result appeared to be an anomaly since eight embryos were found trapped in the silica layer that penetrated the stainless steel mesh. Mortality rates were well within the ASTM standards from FETAX conducted in Petri dishes, while they were slightly higher in the jars. Malformation rates were very similar between controls and experiments conducted using jars. The results showed that neither silica nor blasting sand was toxic and that the supercritical fluid extraction procedure either led to toxicity or concentrated toxic materials from the sand.

6. Soil Sample Exposure Testing Using Aqueous Extraction

a. Procedure.

This procedure was used to extract the contaminants from the soil samples from Eglin AFB. Given the hydrophobicity of the contaminant, it was questionable how useful and reliable this procedure would be in assessing the toxicity of the soil. Therefore, all aqueous extraction tests were considered preliminary.

In a 1:4 ratio of soil to FETAX solution, soil core samples were placed in 250 mL jars and tumbled for 48 hours. After settling overnight in a refrigerator, the samples were centrifuged

for 20 minutes at 8000 rpm. The clear supernatant was decanted into appropriate volumes for FETAX test set-up and renewal. These aliquots were then frozen until needed. Following this extraction procedure, the FETAX test was performed as a standard FETAX test using the frozen samples for daily renewal.

b. Preliminary tests.

Mortality and malformation data summaries of the first test (AE#1) are given in Table 44. The embryos used for this test were not satisfactory. This determination was made due to the fact that Test AE#1, and an unrelated test in the laboratory both had high control mortality rates. Therefore, another test using the same samples was initiated (AE#2). The results from this second test are also given in Table 44.

The next pair of aqueous extraction tests were performed on samples taken from Eglin AFB, but are still considered preliminary as the data were not considered to be reliable. This was because JP-4 is not hydrophilic and was, therefore, not assumed to have dissolved into the water used in the extraction process. The results of these tests (AE#3 and AE#4) are reported in Table 45. The results of AE#4 are much the same as AE#3 with the exception that sample "N" exhibited more toxicity in AE#4. "N" mortality was 47 percent in AE#4, as opposed to only 20 percent in AE#3. The percent malformation was low in all samples with the highest being 16.5 percent in sample "N" from test AE#3.

7. Soil Sample Testing Using Direct Exposure FETAX Tests

a. Procedure.

This testing procedure allowed direct exposure to toxicants in the soil samples without soil contact. The direct exposure test consisted of 5 or 50 mL of soil and approximately 150 mL of FETAX solution mixed in a 250 mL glass jar. An insert was made of a section of glass tubing and stainless steel mesh, with the mesh covering one end of the tube and the other end left open (see Figure 9). The insert was then placed in the jar with the mesh end facing the bottom, and 30 embryos were placed in the insert (see Figure 10). The solution in the jar then flowed freely through the mesh, but the embryos did not contact the soil.

The 250 mL direct exposure jars and Teflon® lined lids were acquired from Baxter®. Stainless steel mesh was acquired from Small Parts, Inc. ®.

There were four FETAX control jar replicates and two replicates of each sample in a standard direct exposure FETAX test. Enough core sample was placed in each jar for a soil depth of approximately 8 mm. Each jar was then filled with FETAX solution. Both the core sample and the FETAX solution were renewed each day.

b. Preliminary Tests.

The first preliminary direct exposure FETAX test (PDE#1) consisted of the same core samples used in the first two aqueous extraction tests (AE#1 and AE#2). Soil was placed in the bottom of the exposure jar to the depth of 8 mm. Due to a shortage of core sample, only one replicate from each sample and the control was renewed daily. The pH of the FETAX solution was monitored daily. All replicates retained a pH of 7.4 to 8.1 for the duration of the test.

Mortality and malformation results are summarized in Table 46. Only one sample, 80 EA 6, showed a high degree of mortality (100 percent). The other samples were 7.0 percent or less.

The next series of preliminary direct exposure tests performed also used samples from Eglin AFB. Fifty mL of soil were used in each exposure jar. These tests are being included in preliminary data, because 50 mL was determined to be too toxic; the volume was reduced to 5 mL. After this, 5 mL samples were analyzed for all pre remediation, during remediation, and post remediation testing.

In preliminary direct exposure FETAX test 2 (PDE#2), the control mortality was around 50 percent in both the no soil control and in the K soil control. The high mortality was partially due to fungus problems and partially due to poor quality eggs. However, some data was still collected from this experiment. The control organisms that were lost to the fungus infestation died at 72 and 96 hours. The rest of the samples had 100 percent mortality at 48 hours. This mortality was not due to fungus. This data was supported by PDE#3. Fungus was still a problem at 72 and 96 hours, but not as severe as in PDE#2. The D.O. was monitored in both of these tests, and all exposure vessels maintained a level of 7.0 ppm throughout both tests. The pH was also monitored and remained between 7.3 and 7.6 in all vessels. A summary of the malformation and mortality data for both tests is given in Table 47. Due to the high degree of mortality and malformation in these two tests, it was decided to reduce the amount of soil used from 50 mL to 5 mL.

Preliminary direct exposure test 4 (PDE#4) involved spiking 300 g of K soil (the remote clean site) with 2.55 mL of JP-4. This was equivalent to the amount of TPH measured in the soil from site S. NRMRL performed all environmental chemical analyses. The results from this test are summarized in Table 48. This test showed that 5 mL of soil would be a better choice over 50 mL for the volume of soil to use during the direct exposure tests.

B. PRE REMEDIATION SOIL TESTING

Samples were taken for FETAX testing from 10 sites. Two sites (S and N) were located in the area of the origination of the JP-4 spill ("ground zero" or GZ) (Figure 32). Two sites (O and B) were located in the nitrate-treated cell (NC). Site E was located in the area of the nitrate-treated cell that was covered with black material to prevent vegetation from growing (NCC). Three sites (R C and G) were located in the control cell (CC) where water was used without nitrate. Site F was located in the area of the control cell that was covered with black material (CCC). Site K was located away from the spill site and was being used as a clean reference site (K-CON). Table 49 summarized site and treatment cells. See Figure 32 for map.

1. Procedure

Three tests were performed on the pre remediation soil samples. These tests were performed using 5 mL of soil in each exposure chamber, changing the soil and the FETAX solution every day. Figure 9 shows the exposure chamber. See "Soil Sample Testing using Direct Exposure FETAX tests" for details regarding the exact procedure.

2. Test Results

The first pre remediation test (PRE-R#1) showed a 20 percent mortality and a 7 percent malformation rate in the control exposure chambers. The results from this test are summarized in Table 50. These results were poor and did not meet ASTM guidelines. The reason for this result was not apparent but the data are present for comparison to other experiments. For direct exposure experiments with 5 mL of soil, the percent mortality and percent malformation should be less than 20 percent. The average percent mortality for the 'ground zero' (Sites S and N) area was 80 percent, and the average percent malformation was 44 percent. For the complete NC (Sites B, O and E), the average percent mortality was 42.7 percent, and the average percent malformation was 6.3 percent. For the complete CC (Sites C, F, G and R), the average percent mortality was 31.0 percent, and the average percent malformation was 4.5 percent. Sites B, C, E, G, and N had significantly different mean lengths from the controls determined by the t-test for grouped observations. These results showed that the soil was most toxic at the site of the spill, and toxicity decreased as distance increased from the spill site. See Figure 32 for specific site locations.

The second pre remediation test (PRE-R#2) was performed in the same manner as PRE#1 and showed a 3 percent mortality and a 17 percent malformation in the control exposure chambers. The results from this test are summarized in Table 51. The average percent mortality for the 'GZ' (Sites S and N) area was 73.5 percent, and the average percent malformation was 100 percent. For the complete NC (Sites B, O and E), the average percent mortality was 75.7 percent, and the average percent malformation was 68 percent. For the complete CC (Sites C, F, G and R), the average percent mortality was 54 percent, and the average percent malformation was 56.25 percent. See Figure 32 for specific site locations. Sites C, F, O, and S had significantly different mean lengths from the controls, as determined by the t-test for grouped observations. This test showed a higher toxicity in the soil than the previous test.

The third pre remediation test (PRE-R#3) showed a 2 percent mortality and a 6 percent malformation in the control exposure chambers. The results from this test are summarized in Table 52. The average percent mortality for the 'GZ' (Sites S and N) area was 100 percent. For the complete NC (Sites B, O and E), the average percent mortality was 63.3 percent, and the average percent malformation was 75 percent. For the complete CC (Sites C, F, G and R), the average percent mortality was 31 percent, and the average percent malformation was 50 percent. See Figure 32 for specific site locations. Sites O, C, B, and F had significantly different mean lengths from the controls, as determined by a t-test. A summary of the three tests is given in Table 53 and Figure 35.

Although the FETAX control values for percent malformation and percent mortality were occasionally above the maximum allowed by ASTM guidelines for Petri dishes, the three tests still yielded acceptable data. The direct exposure jars have not been used extensively and little was known of how well the embryos tolerated changing the soil and solution water each day. It was assumed that controls values would be elevated as a result. The variability between tests was also uncertain, but was assumed to be higher than standard FETAX for the same reasons. Soil samples also contained a higher microbial load to which the embryos were exposed. This probably led to higher mortality and malformation in controls and higher variability.

C. DURING REMEDIATION TESTING

Soil from twelve sites was collected mid way through the remediation process. Site ZG was located in the "ground zero" treatment. Four sites (W, X, Z, and ZA) were located in the NC. Site Y was located in the area of the Nitrate Cell that was covered with black material. Sites ZB, ZC, ZD, ZE and ZF were located in the Control Cell. Site KC was located in the remote clean reference site. Table 49 and Figure 33 summarizes sites and treatment cells. In Figure 33, the Z prefix is deleted from the site designations for clarity.

1. Procedure

Table 54 lists the testing scheme devised for the during remediation samples. All experiments used 5 mL of soil per vessel and had two replicates of 30 embryos each. All samples except KC, Y, ZD, and ZG were divided into four different levels. In these four samples, the top two layers were combined and the bottom two layers were combined. This allowed for more sample during tests of $CaCO_3$. $CaCO_3$ was tested because it was added to sample jars collected before and during remediation. It was thought that this would control pH. Later in testing, it was discovered that pH posed no problem, and $CaCO_3$ was discontinued. direct exposure tests were performed as stated in previous direct exposure sections in this report.

2. Test Results

The results from test D-R#1 are summarized in Table 55. This experiment showed that sites W8 and X11 were toxic to *Xenopus* embryos at 5 mL of soil per vessel. Most of the Z sites were not as toxic although some sites showed significant mortality such as ZC, ZA, and ZE. The mortality in KC1&2 was much less than earlier tests, although still not down to background levels. This experiment showed the relative toxicity of the sites at the upper most level of soil collected for FETAX tests.

The results from test D-R#2 are summarized in Table 56. These results showed that W11, X3, and Z3 caused 100 percent mortality to the embryos at 5 mL per vessel. ZC2 and ZB2 also caused some mortality at this soil volume. The malformation rates in ZB2, ZE4, and the blasting sand were higher than controls. Only two samples reduced growth significantly, ZF4 and the blasting sand. This was determined by comparing the mean length data of the controls with the mean length of data of each sample site separately using a t-test for grouped observations.

The results from test D-R#3 are summarized in Table 57. Controls were well within acceptable limits for this test. These results indicated that samples W9, Z1, ZA2, ZC11, ZD1&2, and ZG10,11 caused 100 percent mortality in the samples tested. Only Y11,10 caused higher than 20 percent malformation rate and sample ZB9 was the only sample to significantly inhibit growth based on the t test. None of the other samples in the test were significantly different at the P=0.05 level from controls using a t-test for grouped observations.

The results from test D-R#4 are summarized in Table 58. These results showed that W10, X2, X1, Z2, ZA3, ZB1, and ZG1,2 caused 100 percent mortality to the embryos at 5 mL soil per vessel. ZG8 caused nearly 100 percent mortality at this soil concentration with only one embryo surviving out of 60. The mortality rates in ZC1, Y1&2, and ZD3,4 had mortality greater than 20 percent. The samples ZE3, and Y1&2 significantly inhibited growth at these soil volumes, because the mean growth data from these samples was significantly lower than that of the controls at the p=0.05 level. Table 49 and Figures 33 and 36 summarize the sites and FETAX results from these sites.

D. POST REMEDIATION TESTING

Soil from 19 sites was collected after completion of remediation. Two sites were located in the ground zero area. Five sites were located in the nitrate-treated cell and three sites were located in the section of the nitrate-treated cell which had been covered with black material to prevent vegetative growth. Five sites were located in the control cell which was treated with water, and three sites were located in the section of the control cell which had been covered with black material to prevent vegetative growth. One site was located in a remote, clean reference site (K-Con). Table 49 and Figure 34 summarizes the sites and the treatment area from which they were collected. In Figure 34 the Z prefix is deleted from the site designations for clarity.

Post remediation soil samples were tested by layers. The samples from each layer were tested twice to confirm the findings. Each test contained two replicates of each sample with 30 embryos each. The volume of soil tested was 5 mL.

The first two tests performed (POST-R#1 and POST-R#2) were on the top layer of soil (Layer 1). The results are given in Tables 59-64. The same degree of mortality and malformation was seen in both tests. Significant differences in mean length between the controls and the samples were seen in ZS, ZT, ZX, and ZY in the first test. No differences were seen in the second test. The data show considerable toxicity in most of the samples. The pH values for both tests were within ASTM guidelines. Significance was determined using a t-test for grouped observations.

The next pair of tests (POST-R#3 and POST-R#4) were performed on the layer of soil directly under the top layer (Layer 2). The results are summarized in Tables 65-70. Mortality and malformation are comparable between the two tests. Test one of layer 2 (POST-R#3) showed significant differences in mean length between the control and the following samples: KD, ZK, ZL , ZT, ZY, and ZZ. Significant differences in mean length between the controls and the samples were seen in ZK, ZL, ZN, ZO, ZP, ZR, ZT, and ZZA in the second test of layer 2 (POST-R#4). The pH values for both tests were within ASTM guidelines. There was almost

100 percent mortality in the sites of cloth covered section of the nitrate cell (ZP, ZR, and ZQ). This may indicate that the nitrate had little effect on the second layer from the surface. High levels of malformation were seen across all sites in the nitrate-treated cell except ZK. This also indicated that the nitrate treatment was not fully effective at reducing the toxicity of the JP-4 spill.

POST-R#5 and POST-R#6 are the two tests performed on Layer 3. Results are summarized in Tables 71-76. Test one of layer 3 (POST-R#5) showed significant differences in mean length between the control and the following samples: KD, ZK, ZL, ZP, ZQ, ZR, ZS, ZV, ZW, ZZ, and ZZA. Significant differences in mean length between the controls and the samples were seen in KD, ZK, ZP, ZQ, ZR, ZS, ZT, ZU, ZV, ZW, ZX, ZZ, ZZA in the second test of layer 3 (POST-R#6). The pH values were within ASTM guidelines for both tests. In the third layer from the surface, malformation and mortality were comparable between the two tests. High degrees of mortality were seen in ZM, ZO, ZT and ZL test #2. ZM and ZO were located in the nitrate cell, but not under the cloth cover. This may suggest that the nitrate was effective in the third layer from the surface. Malformation for layer 3 fell between 10 percent and 30 percent across almost all samples tested. This did not indicate differences between the control and treated cells.

POST-R#7 and POST-R#8 are the tests performed on Layer 4. Results from these tests are detailed in Tables 77-82. Test one of layer 4 (POST-R#7) showed significant differences in mean length between the control and the following samples: ZK, ZN, ZP, ZQ, ZR, ZS ZU, ZV, ZX, ZY, and ZZ. Significant differences in mean length between controls and the samples were seen in ZK, ZN, ZP, ZQ, ZR, ZV, ZW, ZX, and ZY in the second test of layer 4 (POST-R#8). pH values were ASTM guidelines for both tests. In the fourth layer from the surface, malformation and mortality were higher in the second test performed. These differences, however, were consistent for all samples. The levels of difference between tests were comparable. High degrees of mortality were seen in ZL and ZM. ZL was not located in either test cell, but was located near the original spill site (ground zero). ZM was located within the nitrate cell, but not under the cloth cover. This would seem to indicate the nitrate had no effect at reducing the toxicity at the JP-4 spill. Malformation rates for test one were between 5 percent and 12 percent and for test two between 10 percent and 28 percent. The scattered responses in both mortality and malformation seen in these tests do not seem to indicate differences between the control and the treated cell. ZL test #1 mortality was low for layer 3 (Table 71) while it was very high for ZL test #2, layer 3 (Table 72). The reason for this disparity was unclear, but layer 3 marked a transition from relatively nontoxic upper layers to highly toxic low layers and the distribution of toxicants may have been uneven.

Due to suspected contamination from metal flakes from the soil sampling core extractor in certain soil samples from varying layers, two additional pairs of tests were performed. POST-R#9 and POST-R#10 tested JP-4 contaminated samples from layers 1 and 3. These two tests also tested all four layers from site ZGA. This site was added to the FETAX assay after the previous tests were completed. The results from these tests (POST-R#9 and POST-R#10) are given in Tables 83-88. POST-R#11 and POST-R#12 tested contaminated samples from layers 2 and 4. The results from these tests (POST-R#11 and POST-R#12) are given in Tables 89-94. The results showed no significant effect from the metal flake contamination. Therefore, these results were analyzed with the results from the non-contaminated samples. Figures 11 and 12

summarize the percent malformation and percent mortality data by layer for the post remediation samples. Malformation and mortality data were averaged for all tests within a layer and for all sites within a treatment cell. These composite values were then plotted in Figure 37.

E. STATISTICAL DESIGN AND ANALYSIS

The statistics in this project were performed to:

1. Compare the percent mortality, percent malformation, and length data from the three time periods by treatment. This was accomplished by using both a non-parametric ANOVA test and a parametric ANOVA test in the SAS statistical software package.

2. Correlate the percent Mortality, percent Malformation, and Length data with the TPH content at the corresponding sites. These tests showed whether the effects of the embryos to the soil could be strongly correlated to the JP-4 in the soil, or if contaminants affect their growth. These tests were performed using standard correlation tests in the SAS statistical software package.

The first step in statistical analysis involved normalizing the data across all of the separate FETAX tests performed. For each test, the three variables (percent mortality, percent malformation, and length) were divided by the values of the FETAX control jars from that test. If no malformation data was available because of 100 percent mortality, that data point was excluded. After this, the normalized values for each site were averaged between the duplicate tests performed. Three tests were performed on each sample of pre remediation soil one test was performed on each sample of during remediation soil, and two tests were performed on each sample of post remediation soil.

The next step was to average the separate layer values for each variable, as well as BTEX and TPH values, by site. Because the site, or core, is the experimental unit, these layers were considered subsamples. After the subsamples were averaged, the data was arranged in a text file for use in SAS statistical software.

1. Length Data Results

In a two-factor, parametric ANOVA, the variable length was not statistically significant over treatments (0.1911) and was significant over time (0.0003). There was no time by treatment interaction (0.7043). This allowed for the time periods to be compared with the treatment averaged. The mean length of pre remediation samples was 0.88 cm. The mean length of the during remediation samples was 0.98 cm. The mean length of the post remediation samples was 0.92 cm. FETAX solution only control length data for pre remediation, during remediation and post remediation were 0.87, 0.97, and 0.96, respectively. A standard LSD (Lease Significant Difference) test was performed to determine where the significant differences were. Post remediation samples were significantly longer than both the pre remediation and the during remediation samples. There were no significant differences between the pre remediation and the during remediation samples. Figure 13 summarizes the length data by treatment and time period in chart format.

In a non-parametric ANOVA test, the variable length was not significant over treatment (0.1728), but was significant over time (0.0001). There was no treatment by time interaction. This allowed for averaging over treatments to analyze time. The pre remediation samples were significantly smaller than both the during remediation and post remediation samples. The during remediation samples were significantly larger than both the pre remediation and post remediation samples.

There were several explanations for these results. First, these data were collected by three different laboratory technicians which could have caused discrepancies between times. Second, the experiments were conducted at different times of the year. Although we have noticed that embryos are smaller in the fall, we have no statistical data to prove this. However, this could cause some of the differences observed.

The above analysis represents one attempt to compare data across time, treatment and technicians to determine whether remediation was successful as judged by a decrease in growth. Clearly, many problems still exist with this method of analysis, and the analysis should not be considered definitive.

The growth endpoint is the most sensitive FETAX endpoint. Generally, it is analyzed in a concentration-response mode. However, since site samples were analyzed directly, it was not possible to collect concentration-response data. Tables 50-52, 55-58, 61, 62, 69-72, 79, 80, 85, 86, 91, and 92 show that growth was affected by toxicants in Eglin AFB soil samples when these samples were compared to the corresponding FETAX controls or K soil samples. The K site control would be the most appropriate basis of comparison for measurements. Most often an effect was observed in cases where mortality was greater than 50 percent and malformations near 100 percent. This was not true for all samples, but an effect on growth was typically observed. Had the cleanup been more extensive, it would have been possible to observe a decline in growth inhibition, but this would have been the last endpoint to improve due to its sensitivity. Further experimentation must be done to statistically detect improvement due to remediation.

2. Malformation Data Results

In a parametric, two-way ANOVA performed on the percent malformation data, there was no statistical significance among treatments (0.8113), or among times (0.2681). There was also no treatment by time interaction (0.8561). Figure 14 summarizes the percent malformation data by treatment and time in chart format. The mean of the pre remediation samples was 3.28 percent. The mean of the during remediation samples was 5.52 percent. The mean of the post remediation samples was 7.32 percent. Although there were no statistical differences among these data, the trend was for the percent malformation to increase as time passes.

In a non parametric, two-way ANOVA performed on the percent malformation data, there was no statistical significance among treatments (0.4199), but there was statistical significance among times (0.036). There was no treatment by time interaction, allowing us to discuss treatment and time independently. The post remediation samples were significantly higher than both the during remediation samples and the pre remediation samples. If the increase was indeed higher it may have been caused by the nitrate addition to the soil. It may have been toxic to the embryos in an unexpected way. Additionally, the JP-4 metabolites may have been more toxic than the JP-4 parent compounds. This would have caused embryos to be affected through time.

3. Mortality Data Results

In a parametric, two-way ANOVA performed on the percent mortality data, there was significance among treatments (0.0144) and among times (0.0068). There was no treatment by time interaction. This allowed the discussion of the treatment independent of time and the times independent of the treatments. Figure 15 summarized the mean values of percent mortality by treatment and time in chart format. The mean percent mortality value of the control cell was 11.27 percent. The mean value of the covered section of the control cell was 26.03 percent. The mean value of the nitrate cell was 29.42 percent. The mean value of the covered section of the nitrate cell was 23.48 percent. A standard LSD was performed with the following results. The mean value of the nitrate cell was significantly higher than the mean value of the control cell. The mean percent mortality value for the pre remediation samples was 13.82 percent. The mean value for the during-remediation samples was 9.36 percent. The mean value for the post remediation samples was 32.65 percent. The LSD test showed that the post remediation samples were significantly higher than both the during remediation samples and the pre remediation samples.

In a non-parametric, two-way ANOVA performed on the percent mortality, there was statistical significance among both the treatments (0.0001) and the times (0.0001). There was no treatment by time interaction (0.1115), allowing discussion of them independently. Standard LSD tests were performed on both the treatments and the times with the following results. The mean value of the covered control cell was significantly higher than those of the control cell, the nitrate cell, and the covered nitrate cell. The mean value of the post remediation samples was significantly higher than those of the during remediation and pre remediation samples.

The above statistical analyses performed on mortality, malformation, and length data were the first attempt ever to use FETAX data in this manner. It was not very successful. It must be remembered that these analyses pooled all data in a cell for all levels. This may not be the best way to perform this analysis. Considerable site heterogeneity was present which may have complicated the analyses. The analyses are presented here to show what was attempted. Time did not permit further models to be applied or validated. Before trusting these results, a model validation would have to be performed. This was outside the scope of this project.

However, when the data are inspected in the maps (Figures 35, 36, 37), patterns emerge that are insightful even if they cannot be proven statistically. Samples from the ground zero site where the spill occurred (S, N, ZG, ZGA and ZL) all show high mortality, malformation, TPH and BTEX at most levels. The control site (K, KC, KD) always showed low levels of developmental toxicity. It should be observed that toxicity generally declined in the nitrate cell from pre remediation, to during remediation and post remediation samples. Generally, the toxicity was worse towards the spill site (Figure 37, sites ZM, ZQ) and least further away (ZR, ZS). Interestingly, this same pattern was observed for the control cell which received water only. By post remediation, many of the sites, especially those furthest away from the spill, show the + and ◊ symbols on Figures 35-37 indicating less than a 25 percent response for mortality and malformation. Generally, TPH and BTEX values correlated with the toxicity results. The best conclusion which can be reached was that general remediation occurred in both the nitrate and control cells. It was unclear how much the matting helped. However, it seemed that biotic processes at the site were increased and this lead to the cleanup observed.

4. Correlations to TPH.

Standard correlation tests were performed. The correlation coefficient was significant between TPH and percent malformation (0.62029 at p=0.0001), percent mortality (0.71515 at p=0.0001), and length (-0.54335 at p=0.0028). The correlation coefficient was significant between BTEX values and percent malformation (0.65634 at p=0.0001) and percent mortality (0.48838 at p=0.0014). Figure 16 summarized the TPH data by treatment and time in a chart format.

Regressions were performed between the TPH or BTEX values and each variable (percent malformation, percent mortality and length). The log of each value was calculated, and the data was then graphed. Figures 17-31 show these regression lines for each treatment cell and time period.

SECTION IV

ADULT MALE REPRODUCTIVE TOXICITY TESTING

A. INTRODUCTION

The objective for this part of the project was to develop and evaluate a male reproductive toxicity test using the South African clawed frog *Xenopus laevis* with particular emphasis on assessing the toxicity of contaminated soil from Eglin Air Force Base (AFB) in Florida. The reproductive toxicity test described here complements the more developed FETAX assay. *Xenopus laevis* are highly developed vertebrates that share many morphological and physiological similarities with mammals. They are relatively cheap, hardy and fecund. This fecundity allows a large number of gametes and offspring for statistical analysis. The experiment designed below tests the effect of environmental contaminants on gametogenesis in males.

In developing a male toxicity test using *Xenopus*, endpoints were defined and an exposure regimen developed. Tests with known reproductive toxicants were conducted to evaluate the endpoint selection. Then, Eglin AFB samples were tested using the new method. These latter experiments with Eglin AFB soils are presented below.

The first experiment was an initial feeding experiment with weathered JP-4. The next experiment was direct exposure to the contaminated soil samples prior to remediation. Then two feeding studies with SCFE were performed. The final study was a post remediation study, involving direct exposure to the contaminated soil samples.

B. PRELIMINARY TESTING - ORAL EXPOSURE TO JP-4

1. Procedure.

A JP-4 experiment of five control males and five treated males was initiated on November 14, 1993 and terminated on March 1, 1994. Upon receipt, the animals were kept in quarantine for at least one week and inspected for any gross lesions or abnormalities. The animals were branded with liquid nitrogen, indicating a number specific to that frog. This standard laboratory procedure was carried out in all of the following experiments.

The JP-4 was initially injected into pieces of whole beef. The JP-4 was injected into the meat with a disposable 26 gauge ½ inch long needle attached to a Hamilton microliter syringe. A dose was injected into each piece of liver; the dose was dependent on the weight of the frog. The dosage per frog was based on a multiplication factor that was derived from the EPA figure of how much dirt a child eats in one day. This multiplication factor was 0.15 µl/gram frog weight. For example, a 60-gram frog would have received a dose of 9.0 µL of JP-4 per day. The exposure period was 90 days.

During the initial stages of the study, leakage of the JP-4 from the beef liver was noted. It was therefore necessary to investigate alternate vehicles for the JP-4. Several concepts were

investigated; ground liver pellets, Medaka (small fish), and red wiggler worms. Finally, large earthworms (obtained from a local bait shop) were tested and found to be the best available route of oral exposure. The earthworms were determined the best based on comparison of feeding attempts from the various food sources. Due to the reduced size of male *Xenopus*, they received only half an earthworm on a daily basis. When smaller earthworms were available, entire worms were offered to the frogs as food. Whether the food offered on the previous day was consumed was recorded daily. If the body weight of the frog decreased below 85% of the initial body weight, then that frog was to be fed untreated food temporarily, until an increase in body weight could be noted. Body weights were taken on a weekly basis. Water in their exposure chamber was also changed at this time. At the termination of the study, the animals were anesthetized with MS-222 (4%w/v). When the animals were determined to be unresponsive a cut was made at the base of the skull to sever the spinal cord. A necropsy was then performed on the frog, noting any gross lesions or tumors. Each male was sacrificed, and liver, spleen, and testes weights were taken. After the testes were weighed, they were placed in 10 mL of FETAX solution and macerated. The macerated testes solution was placed on a Petroff-Hauser sperm and bacteria counting slide. The slide was observed under 100x magnification and data recorded on sperm numbers, motility, and morphology.

2. Results.

The decrease in body weight which necessitated the investigation into an alternative food source was seen in both the control and treated frogs. The individual weights can be found depicted graphically in Figure 38. The control frogs actually lost weight at a faster pace than the treated frogs. There was no statistical significance between the control and treated at $p=0.05$ for the change in body weight as well as any of the organ weight or sperm parameters. From this table, the data from both the treated and control frogs were very similar in their results to the treatment with JP-4. No trend in the data could be determined from this experiment.

C. SITE TESTING

1. Direct Soil Exposure #1

a. Procedure.

Upon receipt of the animals, the standard quarantine and branding procedures, mentioned previously, were followed. Soil samples were received from Eglin AFB from five different sites. The K site was not located in the spill area and served as a soil control. The treatment sites were O, B, G, and S (Figure 32), and a non-soil group (water only) served as the controls. The S site represented an area of high contamination located near the original spill site. The other sites represented different treatment area sites. For each of the six groups there were three frogs, each frog was housed individually in a 1-gallon glass aquarium. 200 mL of the corresponding soil was placed in the bottom of each glass aquarium. A stainless steel mesh was then placed in the aquarium to prevent the frog and soil from making contact. The steel mesh had Teflon® tubing placed around its perimeter with silicone to protect the frog from the sharp metal edges. Then 2 liters of dechlorinated water was placed in each aquarium and the frog was placed on top of the

mesh screen. The aquaria were covered with a plastic mesh to prevent escape. The aquaria housing the control frogs contained only dechlorinated water along with a stainless steel mesh. Body weights were taken every two weeks, fresh changes of soil and water were also made at this time. Food consumption was noted daily for the food offered on the previous day. The method of termination described for the JP-4 feeding exposure was followed in this experiment as well. The same endpoints were evaluated: liver, lung, spleen, and testes weight, as well as sperm count and morphology. The calculation of sperm count was based on the formula that follows:

$$\frac{\text{\# of sperm counted in 25 squares x dilution factor (30) x 20,000}}{\text{\# of small cells counted (25)}} \qquad \begin{array}{l}20{,}000 = 400 \text{ smaller squares x } 50 \\ \text{(cell depth is 1/50 mm)}\end{array}$$

After termination, the frogs were wrapped in aluminum foil and placed in a freezer pending disposal.

b. Results.

Body weight data from this study can be found in Figure 39. Sperm count data are illustrated in Figure 40. Organ weight data can be found in Figure 41. A frog from each of the K and B groups died before the end of the treatment period and were not used in the statistical analysis. The data did not show statistical significance in any of the endpoints measured with the exception of the total sperm counts. At this endpoint, the B site soil had a significantly lower sperm count than either the untreated controls or the control K site animals. The B-site is located in the uncovered region of the nitrate cell. The S-site had the next lowest sperm count, though not statistically significant. The S-site was located at ground zero of the spill site. The G-site had the number of sperm that was closest to either of the control groups. The ranking of sites in descending order according to their total mean sperm counts are:

K	>	Controls	>	G	>	O	>	S	>	B
Control Soil		Water Only		Uncovered Control Cell		Uncovered Nitrate Cell		Ground Zero		Uncovered Nitrate Cell

The pronounced trend in the sperm count data suggested that the soils were potentially reproductively toxic to males.

2. SCFE Oral Exposure #1

a. Procedure.

Upon receipt of the animals, and after the standard quarantine procedure, five male frogs were placed in each of the following treatment groups: Site O, B, G and S. Site O refers to the uncovered portion of the nitrate cell, (see Figure 32 for specific map information). Site G is located in the uncovered portion of the control cell. Site B is also within the uncovered portion of the nitrate cell. Site S is located at ground zero of the original spill site. A control group was also run containing five frogs. The feeding exposure was similar to that of the pure JP-4 feeding experiment. Earthworms were obtained and injected with an amount determined by the animals body weight, and several other factors. The calculation to determine the dosing factor is as follows:

$$\frac{\text{Ingestion rate (gram soil/day/frog)}}{\text{Amt. of soil processed by SCFE (gram of soil extracted)}} \times \text{Volume of SCFE (µL of SCF)} \times \text{Correction Factor (constant)} \times \text{Frog Wt. (gram)} \times \text{Dilution Factor (constant)}$$

The correction factor referenced above is based on the concentration of TPH of each soil sample where O=12.7; B=6.59; G=6.89; and S=13.79. For example a 60-gram frog would receive 0.15 µL of extract. The ingestion rate was 0.005 g of soil per day per frog. The frogs were housed in 8-quart plastic containers which contained dechlorinated water. The water was changed every 2 weeks, and body weights were also taken at this time. Consumption of the food offered the previous day was recorded.

b. Results.

Due to increased death rates among the test animals decreasing the number per site to three or less, this study was terminated. A possible cause of death could be the ingestion rate of 0.005 grams of soil/day/frog. This rate was adjusted downward in a repeat study, outlined below. Also the animals were maintained in closed containers with little ventilation. The combination of these parameters could have contributed to the increased death rates in this study.

3. SCFE Oral Exposure #2

a. Procedure.

Several changes were made in the second study in consideration of the high death rate seen in SCF Oral Exposure 1. The most significant change was the decrease of the ingestion rate from 1.25 to 5 milligrams of soil/day/frog. Also, the size of the exposure chambers was increased from 8 quarts to 12 quarts. The chambers had a 1 inch hole bored into them to facilitate better air exchange as well as to make feeding less stressful to the animals. Animals were ordered from Xenopus Express and were received on 1-12-95. All males were injected with 0.2 mL Ivermectin (1 mL Ivormec in 10 mL NaCl). Ivermectin is an antibiotic used to treat nematode infections which are common in *Xenopus*. The frogs were treated with Ivermectin as a precautionary measure. Each frog was individually branded by using liquid nitrogen to place the

number on the dorsal surface. Five groups of 5 males were assigned to treatment groups, randomized by block design using body weights as the determining factor. The following sites were tested: O, B, G, and S along with a control group (Figure 32). Body weights were taken weekly, and the water was changed at this time also. The test article was injected into the food source (earthworms) and then offered to the frog as food. This was done on a daily basis and the length of the study was to be 60 consecutive days. To feed the frogs, earthworms were cut in half, each half was injected with the amount of test solution for one frog and placed in a labeled 60 mm plastic petri dish. The dish was designated with the site by using a color-coded system. Each dish was labeled with the animal number on the lid in the appropriate color; black - control, green - O site, orange - B site, blue - G site, and red - S site. The 10 mL stock solutions of the SCF dilutions were made as needed using mineral oil. Glass serum vials were used to contain the stock solutions. A 26 gauge ½ inch long needle was used to draw the SCF out of the vial. A new needle was used every time a draw from the vial was made. The syringes used were 1-mL plastic syringes. A fresh syringe was used for each site. Stock solutions were stored in a refrigerator. Daily food consumption and weekly body weight data were recorded.

The termination procedure was as follows. All animals were anesthetized using a 2 percent MS-222 solution (2 grams/100 mL reagent grade water, approximately 500 mL prepared in a 1000 mL beaker). An incision was made in the cranium to separate the brain's pain centers from the rest of the body. The body cavity of the frog was opened with a series of incisions. The testes were removed and placed in FETAX solution in a plastic petri dish. The time they were placed in the dish was recorded on the top of the dish. The spleen, lungs and liver were then removed and placed in a weigh boat for weighing. The following organs were inspected for gross lesions or other abnormalities: heart, stomach and intestine. Any abnormalities in these or any other organs taken for weight were recorded on the necropsy form. Following necropsy, the remains were wrapped in the aluminum foil lining the tray, labeled with the date, sex, animal number, and site designation and placed in a freezer for later disposal. After the organs were weighed, and their weights recorded on the necropsy form, they were placed in a 10 percent formalin solution for storage. Samples were labeled with the same information as the remains placed in storage. One testis was used for sperm count and malformation data, and the other testis was used for motility analysis. For the sperm count, five individual counts were performed, and statistical analysis was done on the mean counts. The calculation used to determine sperm count was outlined previous (Direct Exposure #1).

b. Results.

For any of the measured endpoints (organ to body weight ratios, sperm counts, sperm abnormalities and change in body weight) there was no noted significance at p=0.05. However, there were several noteworthy necropsy findings. Male No. 89 from the O-site was found to have a right testis that was significantly larger than the left testis. This difference in size did not appear to have any effect on the sperm count or the number of malformations. Also, male No. 87 from the G-site was found to have an accessory spleen as well as malformed testes. The testes were lobulated in shape, with the left testis having a pear like shape, and the right testis had two distinct lobes which were attached. Once again, the apparent abnormality did not seem to have any adverse effect on the number or shape of the sperm within the testes. The body weight data

from this experiment can be found in Figure 42. The sperm count, malformation, and motility data can be found in Figures 43-45, respectively. The organ weight data are found in Figure 46. The O and B sites were located within the uncovered portion of the nitrate cell, the G site was located in the uncovered region of the control cell. The S site was located at ground zero of the spill site. Since there was no statistical significance in the data, interpretation and correlation to site location was not possible. One obvious trend in the data was an increase in sperm malformation in the B and G site soils. The number of animals in each group was increased in future experiments to aid in statistical analysis.

4. Direct Exposure #2

a. Procedure.

The procedure outlined in the Direct Exposure #1 section of the report was followed in this study with only several exceptions. The number of animals per group was increased to seven to allow greater possibility of statistical significance. The amount of soil within each aquarium was decreased from 200 mL to 150 mL to decrease the possibility of direct contact between the frog and the contaminated soil by layering the soil level in each aquaria below the screen. It was found that weighted objects needed to be placed on the plastic mesh covering the aquaria to prevent the frogs from escaping. Several male frogs escaped from their containers during the course of the study. These frogs were recaptured and continued on the study. They did not appear to be harmed. Typically, the frogs escaped in the late evening and were found in the early morning. The soils that were tested were post remediation samples and were from the following sites: ZGA - located at ground zero of the spill site; ZO - in the uncovered portion of the nitrate cell; ZP - located in the covered region of the nitrate cell; ZX - located on the border of the covered and uncovered control cells; and KD - a supposed control site located at a clean remote location (Figure 34). The termination procedure outlined in SCF Oral Exposure #2 was followed in this study. Following termination, the animals were placed in a freezer pending disposal.

b. Results.

The body weight data collected from this experiment can be found in Figure 47. Sperm count, malformation and motility data are illustrated in Figures 48-50. Organ weight data are presented in Figure 51. The termination of the study animals showed no abnormal necropsy findings. The analysis of the body weight change during treatment showed no statistical significance. The organ weight data was analyzed as the percent of the final body weight for each organ. This was a more appropriate analysis because it took into consideration the size of the animal in relation to its body weight before the analysis was done. With a significance level of $p=0.05$, no statistical significance was found in the change in body weight during treatment or in any of the organ:body weight percentages. However, when the number of malformed sperm and sperm counts were analyzed, several significant differences did occur. There was a significantly higher number of malformed sperm from the ZO site as well as the KD site when compared to the water only controls. Recall that the ZO site is located in the uncovered portion of the nitrate cell and that the KD site is supposedly a clean remote site outside of the spill area.

These data infer that there may be something in the KD site that caused the increased malformation rate. The KD site indicated a very low JP-4 level of 5 milligrams per kilogram, and a BTEXTMB of 0.1 milligram per kilogram. A further comparison of JP-4 values maybe found in Table 4. There was also a significantly higher sperm count in the animals from the ZGA group. This does not seem to be treatment related and does not appear to be an indicator of reproductive toxicity. The significantly lower sperm count found in the ZX group when compared to either the control groups or the KD-site control soil group may be an indicator of reproductive toxicity.

D. STATISTICAL ANALYSIS

The analysis of the above data was done using various analyses of variance. Prior to analysis, the homogeneity of the variances of the different sites had to be determined. Levene's test for the homogeneity of variance was selected. This test was done to make sure that differences found significant were between treatment groups rather than within them. Following the homogeneity of variance test, Dunnett's test was selected to compare the control endpoint data to that of the various sites. The analyses for the sperm count data needed to take into consideration the weight of the testes, since there is a correlation between testes size and sperm count. The correlation was taken into consideration when an analysis of covariance was done on the sperm count data. Significance levels reported were all at $p \leq 0.05$.

E. FUTURE STUDY OPTIONS

There are several possibilities for future testing with this method. One of the most informative would be to take the sperm released from the testis for motility evaluation and fertilizing eggs from a hormonally induced female. FETAX could then be done to assess the developmental capabilities of the gametes. Ideally, it would be best to allow the embryos to develop into adult frogs and then breed them to determine their reproductive success, but with the maturation process taking over 1 year, the timetable may not be feasible.

Another option is to look at a different route of exposure. An ideal route would be a subcutaneous injection into the dorsal lymph sac of the frogs. By injecting the test agent directly into the lymph sac, the developing sperm are exposed to the toxicant without any prior detoxification. Advantages include the efficient delivery of nonpolar solvents and direct dosing of known quantities of toxicant. However, this is the least natural method of delivery and suitable only for hazard assessment. In order to minimize trauma, doses would be given every third day of the 60-day exposure period and attempts would quickly be made to cut the exposure time.

In order to properly validate the assay, establishment of a positive control would be necessary. Several attempts were made during the course of the project to find a compound to serve as the positive control, but none were successful. Future testing would definitely need to include the establishment of a positive control compound to run simultaneously with the test compound.

F. CONCLUSION

This project has dealt with the concept of developing a male reproductive toxicity assay utilizing *Xenopus laevis* as the test species. Several different routes of exposure were evaluated, oral and direct. There were samples from pre and post nitrate remediation sites, both covered and uncovered. Although covered nitrate cells were evaluated in the post remediation Direct Exposure Test #2, no covered nitrate cells were initially evaluated. Therefore, it is very difficult to draw any conclusions based on whether the nitrate remediation wass covered or uncovered for a period of time. The covered region of the control cell was not evaluated in either the pre or post remediation studies. In both the pre and post remediation studies, there were no significant effects on body weight or organ weight data at the $p \leq 0.05$ level. However, there were effects seen in sperm count and morphology. In the pre remediation direct soil exposure #1, the B-site was found to have a significantly lower sperm count than either the control group or the K-site control soil group. No significant effects were found in pre remediation studies on soil samples from the control cell or the K-site control soil (G-site was located in the control uncovered control cell). In the post remediation direct exposure test #2, sperm count effects were seen again along with abnormal sperm morphology. The control soil site, K, exhibited a significantly higher number of malformed sperm when compared to the water only control group. The cause of this is unknown, and may warrant further investigation in future reproductive toxicity studies. The covered control cell (G), which had initially shown no reproductively toxic effects in the pre remediation studies, showed a significant decrease in sperm count (ZX) when compared to control animals and the K-control soil site group during post remediation Direct Soil Exposure #2 testing. The significantly lower sperm count seen in the B-site pre remediation was not present at post remediation testing. However, the ZO site, located very close the B-site, showed a significantly higher number of malformed sperm than the control group. At ground zero, there was no evidence of reproductive toxicity in the pre (S-site), or post remediation (ZGA site) studies, but when the ZGA site was tested post remediation, a significant increase in the sperm count was seen when compared to controls. This finding does not seem to be related to the JP-4 spill, since this effect was not seen in any of the other sites. It is difficult to compare sampling sites pre and post remediation (Figures 32 and 34). However, when sperm count data (Figures 40 and 48) is compared against neighboring sampling sites (G and ZX; O and ZP; B and ZO; K and KD; S and ZGA), the post remediation sperm counts were improved in three of four contaminated sites and similar in the control site (K).

In conclusion, the male reproductive toxicity assay under development has given useful information in determining the reproductive toxicity of JP-4. Significant effects were seen in both the pre and post remediation direct exposure tests, indicating that the direct exposure route may be the most promising for future testing.

SECTION V

ADULT FEMALE REPRODUCTIVE TOXICITY TESTING

A. INTRODUCTION

To conserve space, the introduction and dosing schemes presented for the male reproductive toxicity experiments apply to the female experiments as well. Test descriptions and numbers such as Direct Exposure Test #1 are used for both male and female tests and correspond to one another temporally and in terms of the sites tested.

B. PRELIMINARY TESTING

1. Positive Control Testing Using Methoxychlor (DMDT)

a. Background.

Methoxychlor is an organochlorine pesticide that has weak estrogenic properties. It alters female reproductive behavior, modifies the reproductive tract, alters gametogenesis and has proved fetotoxic in mammals (Cummings and Gray, 1987; Swartz and Corkern, 1992; Walters et al., 1993). For these reasons, it was chosen as a potential positive control which should negatively affect reproductive endpoints. It was weak enough that if an effect was observed, it would suggest that the reproductive toxicity tests were quite sensitive.

b. Procedure.

Frogs were bred at the onset of this preliminary test to gather information regarding their clutches and the condition of their gametes. Breeding was performed according to the standard method. Both males and females were injected with 1000 International units. Human Chorionic Gonadotropin (HCG) in the dorsal lymph sac. The frogs were then allowed to breed overnight. Eggs were collected the next morning, and the jelly coats removed with 2.0 percent w/v L-cysteine. Two hundred eggs were randomly selected from each female and sorted into three categories: normal, fertilized, and normally cleaving. The normal category includes eggs that were normal in appearance regardless of whether they are fertilized or not. *Xenopus* females can discharge poorly pigmented and abnormal eggs. The fertilized category provides an assessment of how many eggs were fertilized versus those which were not. Lastly, the number of fertilized eggs were divided into those that were cleaving normally versus those that were not. The creation of these divisions allowed an assessment of where the reproductive fault might lie. Percent of total clutch for these categories was then calculated. The remaining eggs in the clutch were then double sorted following standard FETAX procedures. Two hundred of these double

sorted eggs were then allowed to grow for 96 hours in eight dishes of 25 embryos each. These embryos were treated as standard FETAX test controls. After the 96 hours, embryos were terminated, fixed in 3.0 percent w/v formalin, and the number of malformations determined. Mortality data were also recorded. After the initial breeding, the exposure to the test material began.

One hundred mg/mL of DMDT was dissolved in corn oil. The frogs were fed by injecting the DMDT into worms at does of 0.4, 0.2, and 0.1 mg/g of frog weight. Three control animals and three treated animals were used, one exposed animal for each dosage rate. A dosage schedule was made out and prorated from a 7-day a week to a 5-day a week feeding schedule such that the dose was multiplied by a factor of 1.4. The 0.4- and the 0.2-mg/g frogs were found dead after two days of feeding. Due to the apparent acute toxicity of DMDT in *Xenopus*, altered the dosage was altered to 0.1, 0.05, and 0.01 mg/g. The 0.1- and 0.05-mg/g frogs were found dead after three weeks of exposure. As only one frog remained in our exposure group, one of the controls was dosed and given 0.001 mg/mL of DMDT. The 0.01 mg/g DMDT frog was found dead after 5 weeks of exposure. The second control frog was found dead, apparently from choking on a piece of food. Because testing was done with a pure compound at high concentration, the frogs were bred after a 6-week exposure period instead of an 8-week exposure period.

c. Results.

The results from the positive control test were not complete because of the high instances of adult mortality during exposure. Table 95 gives the reproductive endpoint data collected in both the prior-to-exposure and the after-exposure breeding. Although there was only one exposed animal by the end of treatment, a decline in the percent of viable eggs in the post exposure breeding is seen. This test served as an initial range-finding test using minimal numbers of adults to establish a dosing range for planned experiments. Future experiments will employ far lower doses of DMDT and injection into the dorsal lymph sac as an alternative to oral exposure.

2. JP-4 Oral Exposure Testing

a. Procedure.

This experiment explored the method of direct uptake of the toxicant JP-4 by ingestion. This experiment involved six exposed frogs and 6 control *Xenopus laevis*. These frogs were bred at the onset of this preliminary test to gather information regarding their clutches and the condition of their gametes. Frog breeding and egg collection followed standard protocol as described above. Again, eggs were categorized in the subsequent manner prior to exposure. Two hundred eggs were randomly selected from each female and sorted into three categories: normal, fertilized, and normally cleaving. Percent of total clutch for these categories were then calculated. The remaining eggs in the clutch were then double sorted following standard FETAX procedures. Two hundred of these double sorted eggs were then allowed to grow for 96 hours in eight dishes of 25 embryos each. These embryos were treated as standard FETAX test controls. After the 96 hours, embryos were terminated, fixed in 3.0 percent w/v formalin, and the number of malformations determined using standard ASTM protocol. Mortality data was also recorded. After the initial breeding, the exposure to JP-4 began.

During the first 3 weeks of exposure, the JP-4 was injected into chunks of whole beef liver which were cut to 1/2 inch squares. The injection procedure was performed by attaching a disposable 26-gauge, 1/2 inch needle to a Hamilton microliter syringe. A dose was injected in each piece of liver; the dose was dependent on the weight of the frog. The dosage per frog was based on a multiplication factor that was derived form the EPA estimate of the amount of soil eaten by a child per day. This multiplication factor was 0.15 µL per gram frog weight. Thus, a 60 g frog received 9 µL of JP-4 per day. A week's supply of food was injected once a week; the dose was based on weight of the frog taken the previous day. Some leakage of the JP-4 from the food was noted, and this may have made the food unpalatable. JP-4 leakage was determined by adding oil-red-O dye to the JP-4 then injecting the mixture into the frozen food source. The food source was placed in a beaker of dechlorinated water and watched for a period of time. The color change of the dechlorinated water from clear to pink indicated JP-4 leakage. Food alone did not cause this color change.

Each day the injected food was offered to experimental and control frogs using forceps. If the animal did not take the food immediately, it was left in the tank. The condition of the food was then checked approximately 1 hour after feeding and the results recorded in a spreadsheet. Uneaten food was removed at this time.

All of the female frogs were very receptive to the food during the first 2 weeks. The males had similar results, but the males ate less than the females did. During the third week both the treated males and females exhibited waning appetites. In some instances they avoided the food. As a result, body weights began to drop slightly.

Alternate food sources were investigated to improve the appetites of the frogs. In week 4, ground beef liver was tried. A portion of ground liver was placed in a cocktail ice-cube tray and allowed to freeze with toothpicks placed upright in each cube. Upon freezing, the toothpicks were removed leaving a narrow hole. JP-4 was placed in this hole, and then the frozen chunk of liver was capped with more ground liver and allowed to freeze thoroughly. The dose of JP-4 was based on the weight of the frog. Benefits were that all of the pieces were uniform in size and the preparation process appeared to reduce JP-4 odor. However, two problems resulted. First, the ground liver melted more quickly than whole liver, reducing the amount of time that the frog had to ingest the food. Second, uneaten ground liver had less residual in the tank than whole liver pieces, making the determination of food consumption after one hour difficult.

Small *Medaka* fish were presented as an alternate food source. The frogs ate all of the fish, indicating that live organisms may stimulate feeding. The waste in the tanks was minimum, unlike that left when liver was used as a food source. This method was decided against, however, because of the high cost and the involvement in maintaining this food source. A second live food source, small red wiggler worms, was used for a few days. These were easy to dose and had less JP-4 leakage than the liver. As with the fish, the worms were eaten well, and there was little waste in the tanks. A primary drawback to the use of red wiggler worms was their size; they were too small to maintain the weight of the frogs with the feeding schedule used. In effect, the red wiggler worm diet would have had to be supplemented with liver.

A third live food source, large earthworms, was tested, found successful, and may be the food source of future tests. These organisms were relatively cheap, large enough to maintain the

frog weight and could be cut to size for the smaller male frogs. They were also easy to handle, dose, and maintain.

This experiment was a 60-day exposure, with breeding and reproductive endpoint analysis performed both prior to and after exposure. Frogs were dosed via food injected with JP-4 five days per week. Their aquaria were cleaned weekly.

b. Results.

The exposure was continued past 60 days to 120 days. No reproductive data was collected from this portion of the experiment because of poor performance of the 120 day breeding. Weight data was collected, however, and is detailed in Tables 97, 98, 99 and 100.

All of the animals were bred after treatment. Treated animals appear to have fed slightly less frequently than controls. Table 96 shows the amount of JP-4 ingested per week. Females consumed more JP-4 than males because of their larger size. Most males gained weight during the treatment period while only two animals finished the treatment period at 95 percent of starting weight. For females, one treated female finished at 88 percent of starting weight but most females were heavier than 96 percent of starting weight.

Table 101 shows the reproductive data for the initial breeding, pre-exposure. Table 102 shows the reproductive data for the final breeding, post-exposure. As expected results were somewhat variable for controls. Most of the endpoints showed some decrease in all the controls. The same variability, however, was also seen in the treated animals. If JP-4 was a strong reproductive and developmental toxicant, steep declines in the treated animals would have been recorded. This trend did not occur with pure JP-4. Note that the JP-4 in the soil at the spill sight may have been transformed into reproductive toxicants by microbial action that would make the soil there more toxic than pure JP-4.

C. SITE TESTING

1. SCFE Oral Exposure Test #1

a. Procedure.

Frogs were initially bred according to the standard method described previously. Frogs were housed individually in 1-gallon glass aquaria. The frogs were exposed to the SCFE from Eglin sites via dosed earthworms. The earthworms were injected with a solution of SCFE mixed with light mineral oil, diluted to a 1:100 ratio.

b. Results.

The experiment was terminated early because too many of the adult females died due to SCFE exposure. No data was collected or presented. A second SCFE experiment was conducted using a lower dosing regime. It was concluded that the SCFE was more toxic than a comparable amount of Eglin soil.

2. SCFE Oral Exposure Test #2.

a. Procedure.

There were three major changes to the procedure used above in SCFE experiment #1. First, the frogs were not pre-bred before SCFE exposure. Second, the number of frogs for each exposure group was increased from 3 to 7. Finally, the ingestion rate was scaled down to one fourth of the original dosage (0.005 to 0.00125 grams soil per day per frog). These changes were made in order in improve the development of this test procedure. Frogs were originally pre-bred in order to have a basis on which to judge the post-exposure reproductive results. The pre-breeding success in the females was approximately 60 percent, and because our post-exposure success was approximately the same, it did not help the test analysis to breed the frogs beforehand. The increased number of exposure organisms was necessary to improve statistical interpretations. The ingestion rate was adjusted because of the high toxicity seen in the SCFE #1 experiment.

Seven animals were placed in each treatment group (Control, O, B, G, and S sites). Each frog was liquid nitrogen branded, making them identifiable throughout the experiment. They were housed in 19.5-quart Rubbermaid Storage containers with sealable lids, rather than glass aquaria. Each was filled with approximately 1.5 liters of dechlorinated water. Each container had a one inch hole drilled in the side of the container near the top to provide fresh air as well as use as a port for feeding. This method of undisturbed food presentation was thought to keep the animals as stress-free as possible.

The exposure period lasted 60 days. The start and ending dates were kept to within a three week period. This facilitated the breeding at the end of the test and ensured that it was being conducted to end at exactly 60 days.

The weight of each frog was taken at the beginning of the experiment. The dosage of SCFE was based on this initial weight. Each day the female frogs received one earthworm. The SCFE was administered, diluted with light mineral oil, into the worm via injection. Each day ingestion was recorded by presenting the food and then removing any uneaten material one hour later. The amount of JP-4 consumed was determined by ingestion of the food presented. If any or all of the worm was eaten, it was recorded that the frog ate the entire dose. To help reduce stress and help improve the diet of the animals, their diet was supplemented with Poly-Vi-Sol vitamins by injecting the worms with vitamins three times a week.

The weight of the animals was measured once per week, until the end of the experiment. At the end of the 60 day exposure, the frogs were bred (0.6 mL of HCG for females and 0.4 mL for males). Data were recorded on egg weight, percent normal eggs, percent fertilized eggs, and percent normally cleaving eggs of 200 randomly selected eggs from each female. Another 200 eggs were grown to 96 hours to observe mortality and malformations. The animals were sacrificed following breeding and a necropsy performed to observe any changes in liver weight, spleen weight, lung weight, and ovary weight. The ovary was further dissected to observe ovary stages 1, 2, 3, 4, 5, 6 and necrotic (Dumont et al., 1972). Modifications were made to the protocol so that the quantities measured were more accurate and thorough. The first step in this process was to find a section of the ovary that was flat and could be observed under the Wild M400 Photomacroscope. A section of the ovary approximately 0.5 x 0.15 mm as measured by

the grid in one of the oculars of the macroscope was removed and magnified under 10x power. Once this was accomplished, a thorough count of all the oocytes on the section of ovary was performed. After the larger oocytes were counted, they were teased off the ovary tissue, providing better access to the smaller oocytes underneath and an accurate method of counting all of the oocytes. All the above information was compiled, organized, and subjected to statistical analysis.

b. Results.

Final breeding results were tabulated (Table 103). The necropsy data, also tabulated, is shown in Table 104. There was an increase in the spleen weight when compared to the control with the exception of the S site.

The percentages of the oocytes in Dumont stages 1-6 are detailed in Table 105. In all instances but two (G3 and B3), it was possible to get a complete count of the oocyte populations. For these two individuals, however, it was necessary to estimate the percentage of oocytes because of the morphology of the ovary. In both of these animals the ovary was very irregular and lacked mature oocytes, thus making observation difficult. In both cases they were observed twice to determine whether the initial estimations were accurate. The only other irregularity occurred with site G. Four of the seven individuals of site G had a small portion of their oocytes yellowish in coloration, and this was noted in the 'other' category. This discoloration was not observed in any other animal in any other treatment group.

3. Direct Soil Exposure #1

a. Procedure.

The direct exposure test method provided whole animal exposure to soils from Eglin AFB. The animals were separated from direct contact with the soil by a stainless steel mesh. The frogs were bred at the onset of this test to gather information regarding their clutches and the condition of their gametes. The first direct exposure test was performed between SCFE oral exposures #1 and #2. Because of this, the treatment protocol still included pre-breeding procedures and evaluation. Breeding was performed as done in the standard method, as described previously. Two hundred eggs were randomly selected from each female and sorted into three categories: normal, fertilized, and normally cleaving. Percent of total clutch for these categories were then calculated. The remaining eggs in the clutch were double sorted following standard FETAX procedures. Two hundred of these double sorted eggs were then allowed to grow for 96 hours in eight dishes of 25 embryos each. These embryos were treated as standard FETAX test controls. After the 96 hour, embryos were terminated, fixed in 3.0 percent formalin, and malformations determined. Mortality data was also recorded.

Three frogs were randomly selected for each exposure treatment. There were six different exposures, the control (which received only water and no soil) and five pre remediation sites (O, G, B, S, and K). These sites were selected to be representative of the different remediation treatment areas at Eglin AFB (Figure 32). K site was considered a control site since its location was remote to the JP-4 spill and remediation areas. Frogs were housed individually in 1-gallon aquaria. One hundred and fifty mL of soil were placed in the bottom of these aquaria

and topped by a stainless steel mesh. Two liters of dechlorinated water were then added. The frog was placed on top of the mesh, and a plastic grating was used to cap the aquaria to prevent escape.

b. Results.

Two frogs died over the exposure period, B3 and O3. Initial breeding data are listed in Table 106. Final breeding data is in Table 107. The reproductive endpoints can be ranked in the order of toxicity based on the degree of separation between pre exposure responses and post exposure responses. These rankings from low to high toxicity are:

Female egg weight:

CON > O > G > K > S > B

percent normal embryos from females expose to soil

CON > K > S > B > G > O

The control site had the lowest toxicity in all cases and K site was second lowest in one of the two rankings. Note that pure JP-4 caused no reproductive toxicity in a 90-day exposure and the K site caused little embryo toxicity. Much of the 96 hour data is lacking due to non performance of the females. In many cases, there were no eggs to be fertilized and cultured for four days as seen in Table 107. Controls and the K site showed higher than expected malformation and mortality. The B site showed a large increase in both malformation and mortality. The O site showed a slight decrease in malformation and mortality; however these numbers were still close to the initial breeding data. The embryos from the G site showed an increase in malformation and mortality. The S site individual that gave viable eggs gave mixed results with malformation as slightly increased, and mortality decreased from the initial pre breeding data.

The direct exposure data was statistically analyzed for the day 1 and 96 hour reproductive endpoints. Gabriel's test for variability was used to determine if before and after data were significantly different. Frog weight was used as an independent variable to base the differences of egg weight, percent normal, percent fertilized, percent normally cleaving, and mortality and malformation data. The only significant differences were found in the mortality and malformation data. Site G was significantly different from the control site and the K site for the 96 hour mortality data. The 96 hour malformation data had a p value of 0.0593 and was not interpreted as a significant difference.

Necropsy data were collected upon test termination. The ratio of organ weight to body weight is listed in Table 108. The ovary data is listed in Table 109. Statistical analyses were performed on these data; however, the data are sporadic and difficult to interpret. A larger sample size in future experiments should eliminate some of these problems.

4. Direct Soil Exposure #2

a. Procedure.

The direct exposure method allowed the frogs to be exposed to the soil from Eglin AFB, while not allowing them to come into direct contact with the soil because of the stainless steel mesh that kept each animal above the soil in a 1 gallon aquaria. When the soil samples were received, each jar was labelled with the site and the layer. In order to have enough soil for the exposure series, all post remediation soil layers were thoroughly mixed together. Figure 36 provides a sample site description for these post remediation samples. Breeding followed the same procedure discussed in the SCFE oral exposure #2 in that pre-breeding procedures were not performed.

Seven frogs were randomly selected for each exposure treatment. There were six different exposures, the control (which received only water and no soil) and five sites (ZO, ZGA, ZX, ZP, and KD). Frogs were housed separately in 1-gallon aquaria. One hundred fifty mL of soil were placed in the bottom of these aquaria, then covered by a stainless steel mesh. Two liters of dechlorinated water were then added. The frog was placed on top of the mesh, and a plastic grating was used on top of the aquaria to prevent escape. After the exposure period was over, frogs were bred again, and the reproductive endpoints were calculated. Animals were necropsied, and the oocytes in the ovaries were staged.

b. Results.

Frog #16 in the ZX exposure group died during the experiment. Therefore, there is no data on this frog. The reproductive endpoints from the breeding after exposure are listed in Table 110. Three frogs in the ZP exposure group and one frog in the ZGA exposure group did not produce enough eggs to perform the 96 hour assay. Necropsy data are given in Table 111. These data are in organ to body weight ratio format. Oocyte staging data is listed in Table 112.

Examination of the mean values for clutch weight, percent normal, percent fertile, percent normally cleaving, percent malformation, percent mortality and oocyte staging data for all sites failed to show any real differences between control, KD control, and test sites. When a trend was observed in the data, it was clear that it was not due to high TPH values from the site. For example, the mean percent malformation and percent mortality values for site ZX were 28.8 and 25.5 percent, respectively but ZX had low TPH values (Figure 36). Most of the endpoint values from the other contaminated sites were very close to the control and KD sites. It was concluded that there were no adverse effects on female reproduction resulting from direct exposure to the contaminated soils post remediation. The pre remediation soils caused effects by the direct exposure method but none were seen with the post remediation soils. It is tempting to conclude that the remediation eliminated female reproductive toxicants. While this is possible, the post remediation female reproductive toxicant data does not correlate with the measured TPH in the soil. At this time, it is best to conclude that more assay development needs to be done.

SECTION VI

CONCLUSIONS

A. INTRODUCTION

The project goal was to develop a nitrate-based remediation scheme and prove that it was successful by a combination of environmental chemistry analyses and toxicity tests. The toxicity tests developed would evaluate reproductive and developmental toxicity using the gametes and embryos of the South African clawed frog *Xenopus laevis*. These tests could then be used to monitor the progress of remediation efforts of a JP-4 spill site at Eglin, AFB Florida. The specific objectives were outlined in the Executive Summary.

B. FETAX

At the beginning of the project, the direct exposure, aqueous extraction, and SCF-agarose methods were explored as possible exposure procedures for FETAX. From the very first, it was obvious that the aqueous extraction technique was not suitable for the purposes of this study. Very little toxicity was extracted because the water was unable to dissolve the nonpolar JP-4 in the soil. Conversely, the SCF procedure developed by the NRMRL lab extracted far more toxicity from the soil than could be explained and although tests were performed to determine whether toxicity was coming from the soil or other apparatus, it was not possible to discover the source of toxicity. Some comment was received at a national meeting that this was not a real world scenario. Even before this comment was received, it became apparent that the direct exposure technique using sealed 250 mL wide-mouth jars was the best method of exposure. It main advantage was that the jar could be sealed thereby preventing the escape of volatile organics. Oxygen content and pH was acceptable thoughout the tests. The main disadvantage was the size of the jars and the difficulty in establishing a concentration-response relationship. It was quickly determined that Eglin AFB samples could be reduced from 50 to 5 mL of soil because of the high toxicity that quickly left the soil during the exposure. The primary disadvantage of this system was that it could not be used with the MAS used too assess human health hazards. However, preliminary investigations with JP-4 revealed little bioactivation or deactivation. Therefore, reasonable results could be obtained just with direct exposure and no MAS.

Initial toxicity tests using JP-4 emulsified into 2 percent agarose revealed that JP-4 was developmentally toxic. It had a fairly high TI; it caused severe malformations and it inhibited embryonic growth significantly. Although MAS failed to change the results to a large degree, some slight deactivation was observed, but this probably was statistically significant. Weathered JP-4 still showed high developmental toxicity so toxicity results may be independent of BTEX concentrations. We performed enough experimentation to show that toxicity was present in both fractions.

C. MALE REPRODUCTIVE TOXICITY TEST

For adult exposure, we developed a direct exposure technique which allowed exposure of the animal to contaminated soils. In this experimental design, most absorption of contaminants was through the porous amphibian skin. There was little chance of the contaminant first being detoxified by the digestive system or the liver as in the oral exposure technique. We obtained reproductive toxicity, and we even killed adults using this exposure method. Oral exposure was also explored and the best results were seen when the SCFE was first injected into a fairly large earthworms. The live worms stimulated feeding and even treated animals ate aggressively. We had several experiments terminate abruptly because the adults died, and we had to reduce quantities of SCF on several occasions. Acute toxicity was a very significant problem.

The techniques of inducing sperm release in male *Xenopus* through treatment with HCG have proven unreliable. The best technique was to terminate exposed males, dissect out the testes, and perform studies on them directly. Sperm count and malformation results were always obtained utilizing this method. Because of the presence of other cell types in the macerated testes, the use of a special counting chamber for sperm counts was preferred to spectrophotometric methods. The development of a video system to evaluate the morphology and motility of the sperm was time consuming, but also very beneficial to the project. The system involved a phase contrast microscope with a video camera connected to it. The camera transfers the image to a high resolution video monitor which is connected to a text generator and VCR. The text generator allows the recording of the date and animal number directly on the video tape, along with the sperm images. The computer system contains an image analysis card and video acquisition software. Specific portions of the video tape are stored as image files on the computer using the acquisition software. This software takes 12 "photos" at 0.25 second intervals and stores each photo as a separate file on the computer hard drive. The video card then allows the display of the specific video images on the video monitor. By displaying the first and last images, it is possible to track the motility of the individual spermatozoa. Initially, the computer system required several modifications in order to become fully functional. By the end of the project though, it was possible to obtain motility data relatively efficiently. Although adult toxicity was observed, some reduction in sperm counts and abnormal morphology were seen even at the end of remediation. Some reduction in male reproductive toxicity was observed throughout remediation in all tests cells although the protocol was only fully perfected at the end of the study period.

D. FEMALE REPRODUCTIVE TOXICITY TEST

The female reproductive toxicity test was new in design and untested. It became obvious after the first series of tests that methods which required the frogs to spawn were not very reliable, even when the numbers of breeders were increased. It proved uneconomical and of little use to breed the animals prior to exposure. For females treated by direct exposure to Eglin soil samples, we saw alterations in the proportions of oocytes in the adult ovary. We observed limited effects on the number of grams of eggs laid but

we did not see any effects in embryos resulting from the breeding of treated females and control males. Although our last experiments were the best, we did not see great differences between pre remediation, during and post remediation soil samples. Some improvement was seen. SCFEs proved toxic to adults and reproductively toxic as measured by some of the female endpoints.

E. SITE TOXICITY AND CLEANUP

Correlation analyses showed that FETAX endpoints can be correlated to TPH and BTEX concentrations measured in Eglin soil. It must be remembered that the JP-4 at this site is not only weathered but that other remediation attempts have been performed in the past. Only carefully controlled laboratory studies can correlate TPH and BTEX measurements from freshly produced JP-4 with FETAX endpoints. Refer to Figures 32-37. These maps correlate toxicity to the site of collection. It is readily apparent from Figure 37 that high FETAX mortality and malformation was seen at ground zero (ZGA) site, while the KD control site showed little mortality at most soil layers. Considerable developmental toxicity was seen in a large number of soil layers in both the control and nitrate-treated cells. The presence of the black mat to retard grass growth did not seem to speed remediation. Figure 37 shows fairly good correlation of toxicity to TPH and BTEX concentrations measured in the different soil layers.

When different soil layers are taken into account, we have derived the following order of toxicity (descending) when compared to site:

Mortality: GZ>NC>CCC>NCC>CC>KC
Malformation: GZ>CCC>NC>NCC>CC>KC
Growth: GZ>NCC>NC>CCC>CC>KC

Where GZ=ground zero (sites ZGA & ZL in Figure 37); NC= nitrate cell; NCC= nitrate cell with black mat; CC= control cell; CCC= control cell with black mat and KC= control cell (Site KD in Figure 37).

As GZ was the untreated area where the spill occurred; the toxicity was highest there. As expected, KC was the lowest in toxicity being the site out of the spill area. There was a fairly mixed pattern of toxicity for all of the other sites, indicating that little cleanup was observed. Had toxicity uniformly increased due to nitrate treatment, then the NC series would be more toxic than the CC series. It was not. Had remediation worked as planned, the CC series should have been more toxic than the NC. Some data suggested greater toxicity for the NC series, but different technicians performed the work, and this could have had some effect.

Note that the remediation may have been working when Figures 35-37 are examined for pre, during and post remediation toxicity. Some layers in the post remediation samples were quite toxic but many layers showed reduced developmental toxicity compared to pre and during remediation testing. In the time available, we were

not able to learn how to statistically model the toxicity data through time. It appeared, however, that all areas, including controls, were reduced in toxicity and that nitrate treatment was no better than simple water application. In short, remediation could have been occurring albeit at a slower rate than hoped for. It does seem that FETAX is useful in monitoring the course of remediation and may yet signal when the cleanup has progressed far enough to signal a cessation of remediation. It may also point out which cleanup strategies are counterproductive.

SECTION VII

RECOMMENDATIONS

A. INTRODUCTION

The FETAX developmental toxicity screening protocol was established after 12 years of development. Modification were made to the assay to accommodate soils testing. These included direct exposure, aqueous extraction and SCFE. Part of the SCFE exposure protocol was aqueous exposure to the frog embryos. The 2 percent agarose method was developed to allow nonpolar organics to be exposed.

Conversely, the reproductive toxicity tests employing adult *Xenopus* were brand . new tests. Endpoint and protocol development had to be developed throughout this study along with methods for exposing the adults to the toxicants. Consequently, numerous modifications had to be made to the test protocol.

B. FETAX

At the beginning of the project, the direct exposure, aqueous extraction, and SCF-agarose methods were explored as possible exposure procedures for FETAX. The following recommendations result from our study:

1.) Aqueous extraction is suitable for some contaminants and should be retained.

2.) The size of the direct exposure jars should be reduced as well as the number of test organisms. Experiments should be designed to explore the use of MAS in these jars. The use of antibiotics to control microorganisms should be examined as a way of improving control mortality and malformation.

3.) SCFE is useful for hazard assessment. Additional work should be done to ensure that the apparatus does not add toxicants to the soil. Work should also be done to ensure that the procedure does not alter the composition of the sample.

4.) More research needs to be conducted on how to model toxicity data with contamination levels in these types of projects.

C. ADULT REPRODUCTIVE TOXICITY TESTS

The direct exposure method was most success but direct intra-peritoneal injection of SCFE to assess reproductive toxicity hazard should be explored. Both male and female reproductive toxicity tests showed some reproductive effects from JP-4 despite the adult detoxification system, and blood-gonad barriers not present in embryos. More research with positive controls and protocol is recommended. For the male test, more work needs to be done with the computer analysis of sperm. Commerical systems exist but are too expensive. For the female test, it would be necessary to use two groups of treated frogs. The first would be used for oocyte analysis. The second would be used for gathering data on egg and embryo quality.

TABLE 1. WELL CONSTRUCTION DATA FOR EGLIN AIR FORCE BASE SITE.

Well	Casing Diameter (in)	Elevation of ground surface (ft MSL)	Elevation of TOC (ft MSL)	Stick-up (ft)	Depth to Bottom (ft from G.S.)	Screened Interval (ft from G.S.)	Screen Length (ft)	Grouted Interval (ft from G.S.)
EPA-1	2.0	11.92	13.97	2.05	11.00	1.0 - 11.0	10.0	0.0 - 1.0
EPA-2	2.0	12.79	14.80	2.01	11.00	1.0 - 11.0	10.0	0.0 - 1.0
EPA-3	2.0	12.93	14.89	1.96	11.00	1.0 - 11.0	10.0	0.0 - 3.0
EPA-4	2.0	13.75	15.69	1.94	11.00	1.0 - 11.0	10.0	0.0 - 3.0
EPA-5A	2.0	8.66	10.62	1.97	11.00	1.0 - 11.0	10.0	0.0 - 3.0
EPA-5B	2.0	8.66	10.71	2.05	21.00	11.0 - 21.0	10.0	0.0 - 3.0
EPA-5C	2.0	8.66	10.61	1.95	31.00	21.0 - 31.0	10.0	0.0 - 3.0
EPA83-1	2.0	10.35	12.84	2.49	26.00	20.2 - 25.2	5.0	0.0 - 19.0
EPA83-2	2.0	10.46	12.77	2.31	8.00	2.3 - 7.3	5.0	0.0 - 2.0
EPA83-7	2.0	8.72	10.68	1.96	9.10	3.4 - 8.4	5.0	0.0 - 2.5
PL1	2.0	10.51	12.51	2.00	50.50	? - 50	-	-
PL2	2.0	13.35	15.49	2.14	49.50	? - 50	-	-
PL3	2.0	6.83	8.94	2.11	39.19	? - 40	-	-
R2	6.0	9.78	10.40	0.62	18.50	8.5 - 13.0	4.5	0.0 - 3.0
R3	6.0	10.23	10.90	0.67	18.50	8.5 - 13.0	4.5	0.0 - 3.0
R4	6.0	11.40	11.86	0.46	18.50	8.5 - 13.0	4.5	0.0 - 3.0
B	2.0	10.55	12.14	1.59	13.16	1 - 13?	13?	0?
C	2.0	11.54	13.76	2.22	14.65	1 - 13?	13?	0?
D	2.0	13.16	15.47	2.31	14.47	1 - 13?	13?	0?
E	2.0	13.76	16.10	2.34	14.49	1 - 13?	13?	0?
EA6	2.0	13.91	16.42	2.51	7.91	2.9 - 7.9	5.0	-
PW	4.0	11.26	11.50	0.24	4.32	0.0 - 4.32	4.30	0.0 - 0.2

TABLE 2. PERIODIC WATER QUALITY ANALYSES FOR POL WELLS.

Well	Date	Water Level (ft from TOC)	pH (pH units)	DO (mg/L)	Fe (sol) (mg/L)	Br (mg/L)	Cl (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	NH4-N (mg/L)	o-PO4-P (mg/L)	SO4 (mg/L)	TOC (mg/L)	CH4 (mg/L)
EPA-1	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-1	6/13/94	4.42	6.13	0.2	0.0	150.0	13.4	21.50	0.10	0.05	0.25	11.20	8.8	0.20
EPA-1	8/23/94	4.34	6.07	0.0	4.1	1.6	11.6	0.79	0.05	1.47	0.99	5.59	NA	NA
EPA-2	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-2	6/13/94	5.49	6.64	0.1	0.1	1.1	8.1	0.65	0.05	0.05	0.05	8.52	6.8	0.06
EPA-2	8/23/94	5.19	6.00	0.0	2.0	1.1	9.9	0.05	0.05	0.49	0.02	3.44	NA	NA
EPA-3	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-3	6/13/94	5.58	5.87	0.2	2.2	14.0	8.5	0.05	0.05	2.62	0.05	0.63	71.0	4.92
EPA-3	8/23/94	5.02	5.68	0.0	3.9	5.9	8.9	0.05	0.05	1.86	0.02	0.50	NA	NA
EPA-4	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-4	6/13/94	6.43	6.41	0.0	0.3	1.7	8.8	0.05	0.05	0.05	0.05	8.13	4.6	0.38
EPA-4	8/23/94	5.93	5.80	0.0	6.5	1.2	4.8	0.29	0.05	1.77	0.02	2.94	NA	NA
EPA-5A	2/24/93	3.77	6.15	0.6	7.4	0.5	4.5	0.05	0.05	1.79	0.18	9.17	32.3	9.16
EPA-5A	6/13/94	3.58	5.87	0.0	7.6	5.3	8.4	0.05	0.05	1.69	0.06	5.05	31.7	3.60
EPA-5A	8/23/94	3.11	5.74	0.0	4.8	7.6	6.3	0.05	0.05	2.77	0.24	1.14	NA	NA
EPA-5B	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-5B	6/13/94	3.74	6.37	0.1	11.9	3.0	2.9	0.05	0.05	2.96	0.12	0.76	23.9	9.91
EPA-5B	8/23/94	3.33	5.77	0.0	11.6	8.9	6.0	0.05	0.05	3.41	0.10	1.03	NA	NA
EPA-5C	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-5C	6/13/94	3.66	6.37	0.1	25.5	1.7	3.3	0.05	0.05	0.58	0.05	8.31	3.3	0.20
EPA-5C	8/23/94	3.25	6.08	0.0	18.7	7.4	4.9	0.05	0.05	0.63	0.02	3.30	NA	NA
EPA83-1	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA83-1	6/14/94	7.57	6.19	0.2	NA	0.5	16.2	0.17	0.05	1.35	0.05	1.50	6.6	6.60
EPA83-1	8/27/94	7.42	5.94	0.9	7.1	1.2	20.0	0.05	0.05	1.10	0.10	0.50	5.6	2.65
EPA83-2	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA83-2	6/14/94	4.39	6.39	0.2	NA	0.5	34.7	0.14	0.05	0.37	0.07	3.76	9.6	9.42
EPA83-2	8/27/94	4.11	6.40	0.8	2.0	0.5	10.1	0.05	0.05	0.84	0.02	0.50	9.0	10.70
EPA83-7	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA83-7	6/14/94	6.20	5.32	0.3	NA	1.1	27.7	4.87	0.10	5.09	0.06	39.60	8.3	8.20
EPA83-7	8/27/94	5.62	4.97	1.0	2.1	1.4	6.6	0.05	0.05	0.78	0.04	0.95	7.4	9.78
PL1	2/24/93	5.24	6.64	1.1	4.6	0.5	8.7	0.05	0.05	0.28	0.15	25.30	3.6	0.62
PL1	6/15/94	5.39	6.41	0.6	NA	3.6	4.5	0.05	0.05	0.05	0.05	8.41	3.6	1.75
PL1	8/27/94	4.92	6.25	3.1	10.9	2.8	6.1	0.06	0.05	0.99	0.02	6.93	5.0	2.10

TABLE 2. PERIODIC WATER QUALITY ANALYSES FOR POL WELLS. (CONTINUED)

Well	CO2 (mg/L)	N2 (mg/L)	N2O (mg/L)	BZ (ug/L)	TOL (ug/L)	ETBZ (ug/L)	PXYL (ug/L)	MXYL (ug/L)	OXYL (ug/L)	MESIT (ug/L)	PSCU (ug/L)	TMB (ug/L)	BTEXTMB (ug/L)
EPA-1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-1	186.0	12.50	0.1260	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
EPA-1	NA	NA	NA	0.0	0.0	1.0	1.0	1.2	1.0	0.0	1.0	1.2	6
EPA-2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-2	174.0	13.90	0.0185	0.0	0.0	2.9	5.6	12.0	8.9	26.9	30.8	22.7	110
EPA-2	NA	NA	NA	0.0	1.0	1.3	4.2	5.3	5.7	116.0	83.8	70.6	288
EPA-3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-3	277.0	11.00	0.0000	128.0	931.0	535.0	602.0	1510.0	1080.0	158.0	420.0	226.0	5590
EPA-3	NA	NA	NA	19.4	2100.0	777.0	855.0	2120.0	899.0	93.7	306.0	166.0	7340
EPA-4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-4	294.0	13.00	0.0000	0.0	53.3	145.0	627.0	1100.0	1320.0	343.0	743.0	496.0	4830
EPA-4	NA	NA	NA	24.5	5410.0	1460.0	2370.0	5640.0	4140.0	443.0	1610.0	509.0	21600
EPA-5A	125.0	NA	NA	8.3	2.8	11.7	42.0	80.0	52.4	25.7	47.5	34.6	305
EPA-5A	136.0	12.50	0.0000	0.0	0.0	13.8	35.6	48.4	3.8	16.9	35.9	32.3	187
EPA-5A	NA	NA	NA	1.5	5.4	9.7	42.0	52.3	1.0	16.6	46.0	20.3	195
EPA-5B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-5B	161.0	11.30	0.0000	27.2	1.1	15.4	62.6	61.8	0.0	29.4	157.0	34.3	389
EPA-5B	NA	NA	NA	22.7	1.6	18.6	26.2	43.1	1.0	15.1	204.0	16.1	348
EPA-5C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA-5C	79.9	14.00	0.0000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1
EPA-5C	NA	NA	NA	6.2	1.0	41.7	77.6	53.4	76.5	9.1	78.7	17.6	362
EPA83-1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA83-1	115.0	12.80	0.0000	7.4	39.2	46.8	88.9	122.0	27.8	37.5	214.0	44.6	628
EPA83-1	121.0	14.60	0.0000	2.2	1.0	7.0	12.6	15.1	1.0	16.6	115.0	15.2	186
EPA83-2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA83-2	103.0	9.89	0.0000	19.4	42.0	21.2	65.2	92.0	62.2	35.1	71.7	28.5	437
EPA83-2	239.0	8.48	0.0000	16.1	22.0	4.7	31.0	53.9	57.8	26.5	51.0	24.2	287
EPA83-7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPA83-7	111.0	8.68	2.1000	2.7	5.6	2.8	8.2	13.8	8.5	9.8	23.9	11.3	87
EPA83-7	129.0	7.64	0.0000	1.6	1.7	1.0	3.1	6.9	4.5	11.0	22.9	13.2	66
PL1	82.3	NA	NA	6.1	0.3	2.7	1.9	1.7	0.2	0.2	1.1	0.4	15
PL1	102.0	15.10	0.0000	0.0	1.3	15.7	22.4	26.1	15.9	7.4	28.9	14.3	132
PL1	118.0	12.50	0.0000	23.1	1.0	61.0	108.0	33.3	1.0	7.3	66.2	29.0	330

TABLE 2. PERIODIC WATER QUALITY ANALYSES FOR POL WELLS (CONTINUED)

Well	Date	Water Level (ft from TOC)	pH (pH units)	DO (mg/L)	Fe (sol) (mg/L)	Br (mg/L)	Cl (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	NH4-N (mg/L)	o-PO4-P (mg/L)	SO4 (mg/L)	TOC (mg/L)	CH4 (mg/L)
PL2	2/24/93	6.54	6.35	1.0	2.9	0.5	11.3	0.37	0.05	0.08	0.05	31.20	4.3	4.71
PL2	6/16/94	6.87	5.65	0.6	NA	1.4	4.0	0.05	0.05	0.50	0.05	9.19	1.8	0.91
PL2	8/28/94	6.35	5.62	1.6	1.4	1.1	4.4	0.06	0.05	0.05	0.02	14.50	1.4	0.18
PL3	2/24/93	4.50	6.70	1.0	14.5	0.5	6.2	0.18	0.05	0.92	0.10	6.01	3.8	1.13
PL3	6/14/94	4.38	6.54	0.3	NA	1.1	2.9	0.05	0.05	0.51	0.07	3.92	3.3	1.33
PL3	8/27/94	4.17	6.23	0.7	8.0	0.5	4.4	0.06	0.05	0.35	0.02	1.95	4.2	1.90
R2	3/22/93	1.98	6.10	0.0	NA	1.4	2.0	0.08	0.05	3.44	0.88	1.50	31.0	15.30
R2	6/15/94	1.81	6.12	0.0	NA	10.2	5.5	0.05	0.05	3.11	0.84	0.86	24.8	13.70
R2	8/27/94	1.77	6.09	0.4	4.6	10.9	19.3	0.05	0.05	2.78	0.20	0.50	11.2	3.77
R3	3/22/93	3.40	6.00	0.2	NA	2.6	1.7	0.05	0.05	2.80	0.24	1.94	30.7	6.64
R3	6/15/94	3.37	6.21	0.1	NA	9.3	4.4	0.05	0.05	2.06	0.33	1.57	15.3	8.25
R3	8/27/94	3.17	6.21	0.9	17.1	16.2	8.2	0.06	0.05	2.64	0.18	0.50	12.9	4.09
R4	3/24/93	3.30	6.30	0.3	NA	2.1	6.1	0.05	0.05	0.84	0.52	8.16	3.9	0.65
R4	6/15/94	2.97	6.34	0.1	NA	4.6	5.1	0.05	0.05	2.73	0.37	3.03	13.1	8.63
R4	8/27/94	2.90	5.96	1.0	15.2	17.5	9.1	0.05	0.05	3.13	0.61	4.07	10.5	1.86
B	2/24/93	4.58	6.54	0.6	9.2	0.5	3.5	0.05	0.05	0.60	0.26	2.49	7.1	4.65
B	6/15/94	4.65	6.14	0.1	NA	6.1	3.1	0.05	0.05	0.61	0.18	0.50	13.3	6.47
B	8/27/94	4.29	6.18	1.0	12.3	3.1	9.3	0.05	0.05	2.77	0.22	0.50	16.7	6.57
C	2/24/93	4.90	6.01	0.8	5.3	0.5	0.5	0.16	0.05	1.95	0.25	0.50	24.1	8.51
C	6/15/94	4.74	6.15	0.0	NA	0.9	15.9	0.05	0.05	0.44	0.12	4.53	11.6	0.62
C	8/27/94	4.68	6.24	1.0	5.2	0.5	11.9	0.05	0.05	0.54	0.09	0.50	17.2	1.44
D	2/24/93	6.44	6.26	0.8	8.6	0.5	11.3	0.17	0.05	0.75	0.35	9.79	10.7	7.25
D	6/16/94	6.34	6.16	0.8	NA	0.5	47.2	0.11	0.05	0.05	0.10	6.70	5.3	0.49
D	8/28/94	6.31	6.34	1.2	2.2	0.5	11.0	0.06	0.05	0.17	0.20	1.01	9.8	1.52
E	2/24/93	6.52	6.17	1.3	4.3	0.5	2.2	0.20	0.05	0.45	0.13	4.52	6.2	NA
E	6/16/94	7.00	6.00	0.4	NA	0.9	2.8	0.05	0.05	0.05	0.05	0.89	4.4	3.42
E	8/28/94	6.33	6.05	0.5	2.3	0.5	3.9	0.05	0.05	0.21	0.02	0.50	8.2	9.32
EA6	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EA6	6/16/94	7.26	5.89	0.1	NA	1.0	4.3	0.05	0.05	0.05	0.05	0.67	2.8	9.38
EA6	8/28/94	6.30	5.96	0.4	1.8	0.5	1.5	0.05	0.05	0.24	0.02	0.50	5.0	7.43
PW	2/24/93	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PW	6/16/94	4.32	6.39	3.3	NA	0.5	2.1	0.62	0.05	0.05	0.05	5.58	0.9	0.11
PW	8/27/94	3.13	6.45	6.0	0.1	0.5	2.0	1.23	0.05	0.05	0.02	9.88	2.6	0.01

TABLE 2. PERIODIC WATER QUALITY ANALYSES FOR POL WELLS (CONTINUED)

Well	CO2 (mg/L)	N2 (mg/L)	N2O (mg/L)	BZ (ug/L)	TOL (ug/L)	ETBZ (ug/L)	PXYL (ug/L)	MXYL (ug/L)	OXYL (ug/L)	MESIT (ug/L)	PSCU (ug/L)	TMB (ug/L)	BTEXTMB (ug/L)
PL2	120.0	NA	NA	34.2	0.8	5.4	50.9	263.0	1.3	22.1	62.1	35.7	476
PL2	75.5	15.30	0.0001	0.0	0.0	0.0	2.4	3.2	1.8	1.7	8.2	2.4	20
PL2	67.0	15.10	0.0000	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.6	0.0	3
PL3	72.1	NA	NA	0.7	0.5	1.3	2.4	5.1	1.5	1.0	10.3	1.8	25
PL3	82.6	14.00	0.0000	0.0	0.0	0.0	1.4	2.4	0.0	1.3	8.2	1.5	15
PL3	83.0	13.70	0.0000	0.0	0.0	1.0	4.2	4.3	1.0	2.3	20.9	5.1	39
R2	201.0	6.83	ND	3.6	10.7	152.0	107.0	201.0	34.3	30.9	360.0	47.2	947
R2	157.0	8.85	0.0000	2.7	1.1	57.5	71.7	92.6	15.3	64.1	445.0	45.5	796
R2	163.0	13.30	0.0000	1.0	0.0	8.3	9.3	6.4	7.9	16.7	156.0	24.8	230
R3	137.0	11.40	ND	6.3	2.5	43.0	86.4	132.0	3.9	32.5	103.0	51.4	461
R3	150.0	10.40	0.0000	27.0	5.4	85.5	215.0	250.0	230.0	40.0	248.0	82.5	1180
R3	197.0	13.00	0.0000	5.4	1.4	60.5	126.0	158.0	115.0	63.4	236.0	89.9	856
R4	70.6	14.60	ND	0.0	0.0	0.7	0.8	2.3	0.0	0.0	0.8	0.0	5
R4	143.0	12.80	0.0000	43.4	2.9	59.4	125.0	195.0	26.7	30.5	165.0	62.0	710
R4	180.0	14.00	0.0000	2.9	0.0	9.9	20.3	26.1	19.2	17.5	69.0	43.8	209
B	122.0	NA	NA	28.0	1.2	3.5	9.0	13.4	1.0	1.1	13.3	1.0	72
B	148.0	11.10	0.0000	19.8	10.8	69.3	126.0	232.0	45.8	20.1	106.0	43.8	674
B	209.0	9.14	0.0000	104.0	2.6	301.0	594.0	259.0	7.7	99.3	517.0	146.0	2030
C	118.0	NA	NA	0.4	24.3	147.0	285.0	654.0	146.0	140.0	327.0	181.0	1900
C	146.0	15.40	0.0000	0.0	7.8	24.4	94.7	158.0	299.0	165.0	250.0	225.0	1220
C	163.0	13.50	0.0000	0.0	3.4	10.0	47.8	74.1	141.0	179.0	285.0	217.0	957
D	123.0	NA	NA	4.3	342.0	261.0	730.0	1645.0	494.0	110.0	306.0	153.0	4050
D	147.0	14.00	0.0104	0.0	55.9	72.9	118.0	258.0	168.0	98.8	187.0	95.6	1050
D	192.0	13.90	0.0005	0.0	6.8	46.4	175.0	282.0	243.0	271.0	548.0	395.0	1970
E	NA	NA	NA	2.1	4.1	38.2	199.0	566.0	66.1	85.4	217.0	72.0	1250
E	83.8	13.30	0.0000	0.0	1.2	9.3	75.9	207.0	11.7	42.0	165.0	35.7	548
E	119.0	9.29	0.0000	0.0	3.6	35.2	163.0	596.0	62.5	123.0	338.0	84.3	1410
EA6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EA6	91.6	9.30	0.0000	0.0	0.0	8.5	33.8	72.2	2.4	144.0	234.0	118.0	613
EA6	153.0	10.40	0.0000	0.5	1.1	30.5	133.0	459.0	3.2	131.0	203.0	86.5	1050
PW	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PW	27.3	12.80	0.0032	1.3	14.5	7.5	10.7	21.7	16.8	3.0	7.2	4.0	87
PW	47.0	12.60	0.0055	0.0	4.7	6.1	12.0	9.4	1.0	3.8	14.5	5.3	57

TABLE 3. GEOPROBE WATER DATA FOR EGLIN AIR FORCE BASE SITE PRIOR TO PILOT STUDY, 3/20/93

Area	Sample	Sample	Grade Elev. (ft MSL)	Bot. Screen (ft from GS)	Top Screen (ft from GS)	Bot. Screen (ft MSL)	Top Screen (ft MSL)	pH (pH units)	DO (mg/L)	Fe (sol) (mg/L)	Br (mg/L)	Cl (mg/L)
	80A-1	80A-1	11.08	5.00	3.50	6.08	7.58	6.01	1.1	4.80	0.0	0.0
	80A-2	80A-2	11.08	8.00	6.50	3.08	4.58	6.13	0.3	5.30	0.0	0.0
	80A-3	80A-3	11.08	11.00	9.50	0.08	1.58	6.19	0.5	13.50	0.0	0.0
	80B-1	80B-1	10.92	4.00	2.50	6.92	8.42	5.60	0.5	7.00	0.0	0.0
	80B-2	80B-2	10.92	7.00	5.50	3.92	5.42	6.08	0.3	4.70	0.0	0.0
Proposed Nitrate	80B-3	80B-3	10.92	10.00	8.50	0.92	2.42	6.07	0.3	4.30	0.0	0.0
Treatment	80D-1	80D-1	12.30	5.50	4.00	6.80	8.30	5.80	1.5	2.60	0.0	0.0
Cell	80D-2	80D-2	12.30	8.50	7.00	3.80	5.30	6.20	0.8	0.46	0.9	1.8
	80D-3	80D-3	12.30	11.50	10.00	0.80	2.30	6.20	0.8	1.80	1.4	1.2
	80 E-1	80 E-1	12.28	5.50	4.00	6.78	8.28	5.70	1.1	2.70	2.8	1.7
	80 E-2	80 E-2	12.28	8.50	7.00	3.78	5.28	6.60	1.0	6.15	1.9	1.6
	80 E-3	80 E-3	12.28	11.50	10.00	0.78	2.28	6.70	0.9	1.13	0.5	0.9
	80C-1	80C-1	11.98	5.70	4.20	6.28	7.78	6.20	0.5	2.00	0.0	0.0
	80C-2	80C-2	11.98	8.70	7.20	3.28	4.78	6.40	0.4	2.30	0.0	0.0
	80C-3	80C-3	11.98	11.70	10.20	0.28	1.78	6.40	0.3	2.30	0.0	0.0
	80F-1	80F-1	13.44	5.50	4.00	7.94	9.44	6.10	1.3	0.50	0.3	2.1
	80F-2	80F-2	13.44	8.50	7.00	4.94	6.44	6.51	0.8	0.38	1.4	1.3
Proposed	80F-3	80F-3	13.44	11.50	10.00	1.94	3.44	6.65	1.3	0.09	0.2	1.5
Control Treatment	80G-1	80G-1	13.01	5.70	4.20	7.31	8.81	5.98	0.9	1.18	0.6	1.3
Cell	80G-2	80G-2	13.01	8.70	7.20	4.31	5.81	5.63	0.4	0.37	1.0	2.2
	80G-3	80G-3	13.01	11.70	10.20	1.31	2.81	6.48	0.6	0.97	0.5	1.9
	80H-1	80H-1	12.50	5.70	4.20	6.80	8.30	5.12	1.2	2.40	0.8	1.3
	80H-2	80H-2	12.50	8.70	7.20	3.80	5.30	5.95	0.4	7.50	1.5	9.6
	80H-3	80H-3	12.50	11.70	10.20	0.80	2.30	6.22	0.4	2.30	1.8	4.3
Downgradient	80I-1	80I-1	10.51	5.70	4.20	4.81	6.31	6.10	0.2	4.80	1.4	2.2
of Proposed	80I-2	80I-2	10.51	8.70	7.20	1.81	3.31	5.90	0.3	5.80	2.7	1.8
Nitrate Treatment	80J-1	80J-1	10.12	5.20	3.70	4.92	6.42	6.10	0.1	7.00	1.3	1.5
Cell	80J-2	80J-2	10.12	8.20	6.70	1.92	3.42	6.20	0.1	8.30	2.0	1.3
	80J-3	80J-3	10.12	11.20	9.70	-1.08	0.42	6.20	0.0	11.30	2.0	2.3

TABLE 3. GEOPROBE WATER DATA FOR EGLIN AIR FORCE BASE SITE PRIOR TO PILOT STUDY, 3/20/93 (CONTINUED)

Area	Sample	NO3-N (mg/L)	NO2-N (mg/L)	NH4-N (mg/L)	PO4-P (mg/L)	SO4 (mg/L)	TOC (mg/L)	CH4 (mg/L)	CO2 (mg/L)	N2 (mg/L)	N2O (mg/L)
Proposed Nitrate Treatment Cell	80A-1	0.08	0.05	1.32	0.05	0.00	30.5	3.01	95.0	13.7	0.0048
	80A-2	0.09	0.05	2.74	1.06	0.00	32.2	8.65	138.0	12.5	0.0053
	80A-3	0.09	0.05	3.35	1.43	0.00	29.2	9.72	118.0	11.1	0.0135
	80B-1	0.08	0.05	2.42	0.57	0.00	40.4	3.73	87.0	15.1	0.0070
	80B-2	0.09	0.05	4.74	0.89	0.00	25.1	9.77	113.0	11.0	0.0047
	80B-3	0.09	0.05	3.45	0.29	0.00	24.5	9.00	99.5	12.1	0.0043
	80D-1	0.08	0.05	0.55	0.05	0.00	10.0	0.12	102.0	18.1	0.0026
	80D-2	0.08	0.05	0.76	0.23	0.37	13.2	3.15	70.9	15.8	0.0005
	80D-3	0.08	0.05	1.69	0.05	0.00	22.2	10.20	114.0	10.6	0.0018
	80 E-1	0.05	0.05	1.48	0.14	0.08	57.1	2.86	157.0	14.7	0.0027
	80 E-2	0.10	0.05	2.46	0.05	0.49	40.9	13.70	260.0	6.4	0.0062
	80 E-3	0.09	0.05	0.86	0.05	0.00	12.0	2.80	84.5	15.6	0.0011
Proposed Control Treatment Cell	80C-1	0.18	0.05	0.84	0.05	0.00	26.9	0.95	72.0	18.3	0.0020
	80C-2	0.10	0.05	1.35	0.14	0.00	12.6	9.58	162.0	12.0	0.0023
	80C-3	0.10	0.05	1.13	0.36	0.00	12.1	7.16	181.0	12.4	0.0023
	80F-1	0.17	0.05	1.85	0.05	5.04	20.0	4.61	149.0	12.5	0.0005
	80F-2	0.45	0.05	1.74	0.06	1.77	11.4	5.18	152.0	12.7	0.0004
	80F-3	0.68	0.05	0.05	0.07	4.60	4.2	0.01	43.1	15.3	0.0001
	80G-1	0.09	0.05	0.62	0.05	0.51	8.9	1.00	35.5	16.9	0.0012
	80G-2	0.09	0.05	0.34	0.05	1.31	6.0	2.70	96.9	15.1	0.0004
	80G-3	0.09	0.05	0.40	0.05	0.00	11.5	3.65	84.0	14.6	0.0010
	80H-1	0.08	0.05	0.15	0.05	1.55	6.3	1.01	29.1	15.5	0.0024
	80H-2	0.09	0.05	2.69	0.20	0.28	22.1	10.10	223.0	9.9	0.0075
	80H-3	0.09	0.05	1.23	0.05	1.62	18.1	3.70	229.0	14.4	0.0023
Downgradient of Proposed Nitrate Treatment Cell	80I-1	0.08	0.05	0.85	0.07	0.00	26.8	14.60	108.0	4.4	0.0048
	80I-2	0.09	0.05	2.34	0.27	0.00	29.5	12.20	215.0	6.9	0.0058
	80J-1	0.07	0.05	5.14	1.42	0.00	41.6	14.30	186.0	6.6	0.0070
	80J-2	0.08	0.05	5.28	1.59	0.00	43.2	15.10	204.0	6.1	0.0083
	80J-3	0.08	0.05	4.55	0.43	0.00	53.1	13.00	232.0	7.6	0.0113

TABLE 3. GEOPROBE WATER DATA FOR EGLIN AIR FORCE BASE SITE PRIOR TO PILOT STUDY, 3/20/93 (CONTINUED)

Area	Sample	BZ (ug/L)	TOL (ug/L)	ETBZ (ug/L)	PXYL (ug/L)	MXYL (ug/L)	OXYL (ug/L)	MESIT (ug/L)	PSCU (ug/L)	TMB (ug/L)	BTEXTMB (ug/L)
Proposed Nitrate Treatment Cell	80A-1	19.1	63.1	7.1	19.1	35.1	17.0	204.0	278.0	157.0	800
	80A-2	24.8	15.2	176.0	384.0	761.0	19.8	137.0	289.0	151.0	1960
	80A-3	16.8	10.1	115.0	202.0	405.0	12.8	49.2	144.0	53.5	1010
	80B-1	1.1	205.0	60.5	113.0	232.0	123.0	91.5	221.0	81.6	1130
	80B-2	4.2	39.3	198.0	254.0	324.0	41.8	89.2	284.0	59.6	1290
	80B-3	8.7	34.2	95.0	145.0	140.0	35.6	33.5	184.0	29.9	706
	80D-1	0.0	0.0	0.0	0.5	0.5	0.0	9.7	58.5	32.1	101
	80D-2	3.3	20.4	95.3	190.0	392.0	133.0	67.8	201.0	104.0	1210
	80D-3	17.0	105.0	615.0	906.0	1930.0	516.0	148.0	530.0	255.0	5020
	80 E-1	0.4	165.0	492.0	773.0	1830.0	1210.0	163.0	347.0	217.0	5200
	80 E-2	76.2	12.6	544.0	460.0	812.0	81.3	71.0	416.0	76.3	2550
	80 E-3	1.0	2.4	18.8	26.6	53.0	20.9	9.1	21.8	10.2	164
Proposed Control Treatment Cell	80C-1	0.0	11.6	8.2	36.6	64.8	51.8	207.0	480.0	308.0	1170
	80C-2	16.9	9.9	63.4	178.0	328.0	14.0	187.0	549.0	286.0	1630
	80C-3	2.6	9.2	29.2	110.0	138.0	12.4	203.0	534.0	262.0	1300
	80F-1	0.0	7.4	16.3	29.0	49.3	207.0	48.9	16.7	103.0	478
	80F-2	0.0	209.0	640.0	933.0	2110.0	1610.0	226.0	601.0	254.0	6580
	80F-3	0.0	2.9	13.1	22.8	48.8	35.8	8.4	17.1	10.4	159
	80G-1	0.0	24.1	26.4	251.0	405.0	700.0	154.0	180.0	261.0	2000
	80G-2	0.3	970.0	590.0	1190.0	2550.0	1730.0	336.0	934.0	353.0	8650
	80G-3	0.0	14.3	26.7	210.0	581.0	47.1	109.0	223.0	118.0	1330
	80H-1	0.0	2.7	4.4	13.7	26.5	14.2	33.4	66.0	40.4	201
	80H-2	29.4	941.0	350.0	691.0	1520.0	153.0	233.0	694.0	287.0	4900
	80H-3	100.0	5150.0	1700.0	3120.0	6750.0	5480.0	327.0	1090.0	406.0	24100
Downgradient of Proposed Nitrate Treatment Cell	80I-1	54.8	61.9	129.0	340.0	678.0	133.0	15.0	45.7	30.2	1490
	80I-2	303.0	4.7	271.0	512.0	515.0	3.6	73.5	483.0	116.0	2280
	80J-1	5.8	1.6	202.0	192.0	182.0	0.8	33.3	357.0	69.0	1040
	80J-2	9.0	1.7	229.0	213.0	63.2	0.6	38.0	393.0	38.8	986
	80J-3	82.2	6.8	296.0	157.0	41.9	4.0	13.3	373.0	19.9	994

TABLE 4. CUMULATIVE GC/MS DATA FOR ELGIN AIR FORCE BASE SOIL CORES.

Sample ID	Interval (ft)	Parameter	BZ	TOL	ETBZ	PXYL	MXYL	OXYL	MESIT	PSCU	TMB	BTEXTMB	JP4
80A	10.3	Sum (mg-ft/kg)	0.06	0.01	1.72	5.16	11.52	0.01	5.78	13.72	4.62	42.6	2070
		Mean (mg/kg)	0.01	0.00	0.17	0.50	1.12	0.00	0.56	1.33	0.45	4.1	201
		Cum Mass (g/m2)	0.04	0.01	0.97	2.93	6.53	0.01	3.28	7.78	2.62	24.1	1174
80B	12.0	Sum (mg-ft/kg)	0.03	0.05	0.06	0.09	0.13	0.01	0.21	0.79	0.15	1.5	504
		Mean (mg/kg)	0.00	0.00	0.00	0.01	0.01	0.00	0.02	0.07	0.01	0.1	42
		Cum Mass (g/m2)	0.01	0.03	0.03	0.05	0.07	0.01	0.12	0.45	0.08	0.9	286
80C	7.0	Sum (mg-ft/kg)	0.00	0.02	0.06	0.23	0.37	0.23	5.24	6.42	3.93	16.5	2719
		Mean (mg/kg)	0.00	0.00	0.01	0.03	0.05	0.03	0.75	0.92	0.56	2.4	388
		Cum Mass (g/m2)	0.00	0.01	0.03	0.13	0.21	0.13	2.97	3.64	2.23	9.3	1541
80D	10.0	Sum (mg-ft/kg)	0.02	0.15	0.39	0.59	1.49	0.74	0.14	1.24	0.29	5.1	712
		Mean (mg/kg)	0.00	0.01	0.04	0.06	0.15	0.07	0.01	0.12	0.03	0.5	71
		Cum Mass (g/m2)	0.01	0.08	0.22	0.34	0.84	0.42	0.08	0.70	0.16	2.9	404
80E	10.1	Sum (mg-ft/kg)	0.15	0.05	3.20	4.57	11.59	2.71	4.65	7.10	2.02	36.0	2932
		Mean (mg/kg)	0.01	0.01	0.32	0.45	1.15	0.27	0.46	0.70	0.20	3.6	290
		Cum Mass (g/m2)	0.08	0.03	1.81	2.59	6.57	1.54	2.64	4.02	1.15	20.4	1662
80F	6.6	Sum (mg-ft/kg)	0.00	0.39	3.97	7.41	16.38	14.21	8.45	13.95	6.79	71.6	3185
		Mean (mg/kg)	0.00	0.06	0.60	1.12	2.48	2.15	1.28	2.11	1.03	10.8	483
		Cum Mass (g/m2)	0.00	0.22	2.25	4.20	9.29	8.05	4.79	7.91	3.85	40.6	1805
80G	7.0	Sum (mg-ft/kg)	0.00	0.68	1.49	11.37	17.47	28.57	19.58	20.94	12.09	112.2	6639
		Mean (mg/kg)	0.00	0.10	0.21	1.62	2.50	4.08	2.80	2.99	1.73	16.0	948
		Cum Mass (g/m2)	0.00	0.39	0.85	6.45	9.90	16.19	11.10	11.87	6.86	63.6	3764
80H	6.5	Sum (mg-ft/kg)	0.05	1.91	0.39	0.59	1.43	0.53	0.22	0.65	0.22	6.0	32
		Mean (mg/kg)	0.01	0.29	0.06	0.09	0.22	0.08	0.03	0.10	0.03	0.9	5
		Cum Mass (g/m2)	0.03	1.08	0.22	0.33	0.81	0.30	0.13	0.37	0.13	3.4	18
80I	3.5	Sum (mg-ft/kg)	0.11	1.26	1.10	3.02	6.10	8.81	0.24	1.70	0.37	22.7	4210
		Mean (mg/kg)	0.03	0.36	0.32	0.86	1.74	2.52	0.07	0.49	0.10	6.5	1203
		Cum Mass (g/m2)	0.06	0.72	0.63	1.71	3.46	4.99	0.14	0.96	0.21	12.9	2387
80J	4.0	Sum (mg-ft/kg)	0.01	0.01	0.29	0.17	0.16	0.00	0.11	0.93	0.12	1.8	28
		Mean (mg/kg)	0.00	0.00	0.07	0.04	0.04	0.00	0.03	0.23	0.03	0.5	7
		Cum Mass (g/m2)	0.01	0.01	0.16	0.09	0.09	0.00	0.06	0.53	0.07	1.0	16
80K	7.5	Sum (mg-ft/kg)	0.00	0.00	0.01	0.09	0.25	0.06	0.03	0.07	0.04	0.5	38
		Mean (mg/kg)	0.00	0.00	0.00	0.01	0.03	0.01	0.00	0.01	0.01	0.1	5
		Cum Mass (g/m2)	0.00	0.00	0.01	0.05	0.14	0.03	0.01	0.04	0.02	0.3	21
80L	6.0	Sum (mg-ft/kg)	0.00	0.18	0.17	0.41	0.90	0.75	0.29	0.62	0.26	3.6	45
		Mean (mg/kg)	0.00	0.03	0.03	0.07	0.15	0.12	0.05	0.10	0.04	0.6	8
		Cum Mass (g/m2)	0.00	0.10	0.10	0.23	0.51	0.42	0.17	0.35	0.15	2.0	26

TABLE 4. CUMULATIVE GC/MS DATA FOR ELGIN AIR FORCE BASE SOIL CORES (CONTINUED)

Sample ID	Interval (ft)	Parameter	BZ	TOL	ETBZ	PXYL	MXYL	OXYL	MESIT	PSCU	TMB	BTEXTMB	JP4
80M	4.0	Sum (mg-ft/kg)	0.00	0.01	0.05	0.09	0.21	0.00	0.06	0.16	0.08	0.7	6
		Mean (mg/kg)	0.00	0.00	0.01	0.02	0.05	0.00	0.02	0.04	0.02	0.2	2
		Cum Mass (g/m2)	0.00	0.01	0.03	0.05	0.12	0.00	0.03	0.09	0.04	0.4	4
80N	6.5	Sum (mg-ft/kg)	0.06	20.88	45.78	62.86	159.79	97.19	40.38	125.72	38.11	590.8	16228
		Mean (mg/kg)	0.01	3.21	7.04	9.67	24.58	14.95	6.21	19.34	5.86	90.9	2497
		Cum Mass (g/m2)	0.03	11.84	25.95	35.63	90.59	55.10	22.89	71.27	21.61	334.9	9200
80O	18.5	Sum (mg-ft/kg)	0.12	0.89	3.31	5.29	9.47	0.39	12.69	26.61	7.93	66.7	5773
		Mean (mg/kg)	0.01	0.05	0.18	0.29	0.51	0.02	0.69	1.44	0.43	3.6	312
		Cum Mass (g/m2)	0.07	0.50	1.88	3.00	5.37	0.22	7.19	15.09	4.50	37.8	3273
80P	19.0	Sum (mg-ft/kg)	0.14	5.22	1.32	3.00	6.93	2.29	3.54	3.94	1.76	28.1	2582
		Mean (mg/kg)	0.01	0.27	0.07	0.16	0.36	0.12	0.19	0.21	0.09	1.5	136
		Cum Mass (g/m2)	0.08	2.96	0.75	1.70	3.93	1.30	2.01	2.23	1.00	16.0	1464
80Q	10.0	Sum (mg-ft/kg)	0.00	0.00	0.05	0.24	0.65	0.04	0.05	0.11	0.06	1.2	24
		Mean (mg/kg)	0.00	0.00	0.01	0.02	0.07	0.00	0.00	0.01	0.01	0.1	2
		Cum Mass (g/m2)	0.00	0.00	0.03	0.14	0.37	0.02	0.03	0.06	0.04	0.7	13
80R	7.5	Sum (mg-ft/kg)	0.01	0.07	0.44	3.70	5.23	9.33	20.51	18.12	19.68	77.1	7036
		Mean (mg/kg)	0.00	0.01	0.06	0.49	0.70	1.24	2.74	2.42	2.62	10.3	938
		Cum Mass (g/m2)	0.00	0.04	0.25	2.10	2.96	5.29	11.63	10.27	11.16	43.7	3989
80S	8.0	Sum (mg-ft/kg)	0.03	4.41	57.77	82.03	168.39	90.78	49.64	114.35	53.64	621.0	19967
		Mean (mg/kg)	0.00	0.55	7.22	10.25	21.05	11.35	6.21	14.29	6.71	77.6	2496
		Cum Mass (g/m2)	0.02	2.50	32.75	46.50	95.46	51.46	28.14	64.82	30.41	352.1	11319
80T	7.0	Sum (mg-ft/kg)	0.01	0.00	0.01	0.41	1.04	0.07	0.10	0.23	0.12	2.0	9
		Mean (mg/kg)	0.00	0.00	0.00	0.06	0.15	0.01	0.01	0.03	0.02	0.3	1
		Cum Mass (g/m2)	0.01	0.00	0.01	0.23	0.59	0.04	0.06	0.13	0.07	1.1	5
80U	11.4	Sum (mg-ft/kg)	0.26	65.28	77.47	99.93	228.04	143.86	73.53	178.18	72.69	939.2	24591
		Mean (mg/kg)	0.02	5.73	6.80	8.77	20.00	12.62	6.45	15.63	6.38	82.4	2157
		Cum Mass (g/m2)	0.15	37.01	43.92	56.65	129.27	81.55	41.69	101.01	41.21	532.5	13941
80V	8.4	Sum (mg-ft/kg)	0.00	11.04	7.98	20.21	40.82	33.31	26.33	36.43	22.63	198.8	5307
		Mean (mg/kg)	0.00	1.31	0.95	2.41	4.86	3.97	3.13	4.34	2.69	23.7	632
		Cum Mass (g/m2)	0.00	6.26	4.53	11.46	23.14	18.89	14.93	20.65	12.83	112.7	3009
80KC	7.3	Sum (mg-ft/kg)	0.00	0.02	0.03	0.18	0.48	0.00	0.04	0.09	0.04	0.9	8
		Mean (mg/kg)	0.00	0.00	0.00	0.02	0.07	0.00	0.01	0.01	0.01	0.1	1
		Cum Mass (g/m2)	0.00	0.01	0.02	0.10	0.27	0.00	0.02	0.05	0.02	0.5	4
80W	7.5	Sum (mg-ft/kg)	0.00	0.03	0.06	0.61	0.56	0.89	14.95	19.19	15.38	51.7	6337
		Mean (mg/kg)	0.00	0.00	0.01	0.08	0.07	0.12	1.99	2.56	2.05	6.9	845
		Cum Mass (g/m2)	0.00	0.02	0.03	0.34	0.32	0.51	8.48	10.88	8.72	29.3	3592

TABLE 4. CUMULATIVE GC/MS DATA FOR ELGIN AIR FORCE BASE SOIL CORES. (CONTINUED)

Sample ID	Interval (ft)	Parameter	BZ	TOL	ETBZ	PXYL	MXYL	OXYL	MESIT	PSCU	TMB	BTEXTMB	JP4
80X	7.5	Sum (mg-ft/kg)	0.37	0.48	132.29	150.28	374.07	1.08	415.72	254.76	97.85	1426.9	33513
		Mean (mg/kg)	0.05	0.06	17.64	20.04	49.88	0.14	55.43	33.97	13.05	190.3	4468
		Cum Mass (g/m2)	0.21	0.27	75.00	85.19	212.06	0.61	235.67	144.42	55.47	808.9	18998
80Y	8.4	Sum (mg-ft/kg)	0.00	0.03	0.56	1.25	2.31	1.25	2.17	5.84	2.11	15.5	3230
		Mean (mg/kg)	0.00	0.00	0.07	0.15	0.27	0.15	0.26	0.69	0.25	1.8	385
		Cum Mass (g/m2)	0.00	0.02	0.32	0.71	1.31	0.71	1.23	3.31	1.20	8.8	1831
80Z	7.4	Sum (mg-ft/kg)	0.01	0.06	7.02	11.64	21.50	0.33	18.16	34.85	14.36	107.9	7469
		Mean (mg/kg)	0.00	0.01	0.95	1.57	2.91	0.04	2.45	4.71	1.94	14.6	1009
		Cum Mass (g/m2)	0.01	0.04	3.98	6.60	12.19	0.19	10.29	19.76	8.14	61.2	4234
80ZA	7.5	Sum (mg-ft/kg)	0.00	0.02	0.00	0.01	0.03	0.06	2.64	1.57	1.26	5.6	4805
		Mean (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.01	0.35	0.21	0.17	0.7	641
		Cum Mass (g/m2)	0.00	0.01	0.00	0.01	0.02	0.03	1.50	0.89	0.71	3.1	2724
80ZB	7.5	Sum (mg-ft/kg)	0.00	0.02	0.04	0.22	0.29	0.05	1.87	2.34	2.58	7.4	1845
		Mean (mg/kg)	0.00	0.00	0.01	0.03	0.04	0.01	0.25	0.31	0.34	1.0	246
		Cum Mass (g/m2)	0.00	0.01	0.02	0.12	0.16	0.03	1.06	1.33	1.46	4.2	1046
80ZC	7.5	Sum (mg-ft/kg)	0.00	0.04	1.15	8.43	13.64	7.64	21.93	32.94	16.17	101.9	6564
		Mean (mg/kg)	0.00	0.01	0.15	1.12	1.82	1.02	2.92	4.39	2.16	13.6	875
		Cum Mass (g/m2)	0.00	0.02	0.65	4.78	7.73	4.33	12.43	18.67	9.17	57.8	3721
80ZD	7.5	Sum (mg-ft/kg)	0.00	0.02	0.01	0.04	0.03	0.02	1.99	0.78	0.47	3.4	2317
		Mean (mg/kg)	0.00	0.00	0.00	0.01	0.00	0.00	0.27	0.10	0.06	0.5	309
		Cum Mass (g/m2)	0.00	0.01	0.01	0.02	0.02	0.01	1.13	0.44	0.27	1.9	1314
80ZE	7.5	Sum (mg-ft/kg)	0.00	0.00	0.01	0.06	0.07	0.07	0.23	0.27	0.20	0.9	9
		Mean (mg/kg)	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.04	0.03	0.1	1
		Cum Mass (g/m2)	0.00	0.00	0.00	0.03	0.04	0.04	0.13	0.15	0.11	0.5	5
80ZF	7.5	Sum (mg-ft/kg)	0.00	0.00	0.00	0.00	0.01	0.00	0.20	0.13	0.17	0.5	8
		Mean (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.02	0.1	1
		Cum Mass (g/m2)	0.00	0.00	0.00	0.00	0.01	0.00	0.11	0.07	0.10	0.3	4
80ZG	7.5	Sum (mg-ft/kg)	0.02	0.17	48.60	88.10	196.16	0.68	83.55	175.45	74.11	666.8	23626
		Mean (mg/kg)	0.00	0.02	6.48	11.75	26.15	0.09	11.14	23.39	9.88	88.9	3150
		Cum Mass (g/m2)	0.01	0.10	27.55	49.94	111.20	0.39	47.37	99.46	42.01	378.0	13394
80ZH	5.5	Sum (mg-ft/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	1
		Mean (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0
		Cum Mass (g/m2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	1
80ZI	5.0	Sum (mg-ft/kg)	0.00	0.03	0.00	0.05	0.03	0.03	9.81	0.24	4.00	14.2	3299
		Mean (mg/kg)	0.00	0.01	0.00	0.01	0.01	0.01	1.96	0.05	0.80	2.8	660
		Cum Mass (g/m2)	0.00	0.01	0.00	0.03	0.01	0.02	5.56	0.13	2.27	8.0	1870

TABLE 5. DISTRIBUTION OF COMPOUND CLASSES IN CONTAMINATED CORES, ELGIN AIR FORCE BASE.

Core	Alkanes	Aromatics	Cycloalkanes	Alkenes	PAHs	Other	JP4
	(wt %)	(wt %)	(wt %)	(wt %)	(wt %)	(wt %)	(mg/kg)
JP-4	57.79	15.89	18.87	1.90	4.61	0.93	-
JP-4	59.30	17.70	17.50	0.94	3.65	0.95	-
Mean	58.55	16.80	18.19	1.42	4.13	0.94	
Stdev	1.07	1.28	0.97	0.68	0.68	0.01	
80A12	63.06	14.14	14.24	1.05	4.41	3.03	1850
80B12	69.16	9.19	16.21	0.36	2.69	2.27	375
80C3	67.77	12.13	13.51	0.32	4.84	1.34	926
80 E15	60.99	13.27	20.59	0.36	3.85	0.98	3270
80F15	62.16	18.13	10.93	1.74	5.50	1.47	2570
80G3	66.50	13.18	11.41	3.19	4.08	1.59	4230
80N2	61.43	17.23	11.32	0.99	7.42	1.54	3370
80O13	60.16	12.34	15.31	1.62	8.96	1.61	10700
80P15	62.69	14.02	12.34	2.52	7.30	1.13	2350
80R9	60.42	13.27	14.58	1.50	8.36	1.87	7720
80S9	61.64	15.41	11.99	1.29	7.68	2.00	11700
80U2	66.50	12.80	15.12	0.00	2.02	3.49	15500
80V1	68.70	13.70	12.00	0.74	0.21	4.66	3340
Mean	63.94	13.75	13.81	1.21	5.18	2.08	
Stdev	3.29	2.26	2.67	0.92	2.66	1.06	
80D12	75.10	9.97	9.38	0.68	2.60	2.16	595
80I4	82.96	1.71	11.61	0.97	1.19	1.56	2010
80H7	0.00	100.00	0.00	0.00	0.00	0.00	12
80J6	93.80	0.00	6.25	0.00	0.00	0.00	8
80L3	12.77	59.81	0.00	0.00	11.34	13.44	18
80M2	0.00	18.20	0.00	0.00	81.80	0.00	3

TABLE 6. KEY TO TEST ABBREVIATION NAMES

Section Title	Test abbreviation
JP-4 Exposure Testing Using Agarose	JP-4#*
Background Testing for SCF FETAX Tests	JCA#*
Supercritical Fluid FETAX Tests	SCF#*
Asessing the Toxicity of the SCFE Process	SAND#*
Soil Sample Exposure Testing using Aqueous Extraction	AE#*
Testing JP-4 and Weathered JP-4	WJP-4#*
Soil Sample Testing Using Direct Exposure FETAX Tests	PDE#*
Pre Remediation Soil Testing	PRE-R#*
During Remediation Soil Testing	D-R#*
Post Remediation Soil Testing	POST-R#*

TABLE 7. SETUP OF CONCENTRATIONS FOR FETAX TEST 1
(JP-4#1--JP-4 AND AGAROSE)

% JP-4	mL agarose	mL FETAX	mL JP-4
0 (Control)	7	1.0	0.0
0.125	7	0.99	0.01
1.25	7	0.9	0.10
3.125	7	0.75	0.25
6.25	7	0.5	0.5
12.5	7	0.0	1.0

TABLE 8. MORTALITY DATA FOR TEST 1 (JP-4#1--JP-4 AND AGAROSE)

JP-4 (%)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	3	0	0	0	1	0	20.0	0.0	
Control	0	0	1	0	0	1	1	1	10.0	10.0	10.0
0.125	0	0	0	0	0	0	0	0	0.0	0.0	0.0
1.25	1	0	0	0	0	0	0	0	5.0	0.0	3.0
3.125	1	1	0	4	0	0	1	0	10.0	25.0	18.0
6.25	0	0	2	0	18	1	0	0	100.0	5.0	53.0
12.5	1	1	19	0	0	4	0	14	100.0	95.0	98.0

TABLE 9. MALFORMATION DESCRIPTIONS AND QUANTITIES FROM TEST 1, (JP-4#1--JP-4 AND AGAROSE)

Concentrations (% JP-4)	Controls				0.125		1.25		3.125		6.25		12.5					
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe				1	3	3	5					2						
Stunted																		
Gut	2	2	1	2			1	1	1			3		1				
Edema																		
Multiple																		
Cardiac																		
Abdominal												1						
Facial												1						
Cephalic																		
Optic																		
Tail									1									
Notochord								1		8		9		1				
Fin																		
Face		1																
Eye		1										1						
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	2	2	1	3	3	3	7	2	2	8	0	11	0	1				
No. Scored	16	20	18	18	20	20	19	20	18	15	0	19	0	1				

TABLE 10. RESULTS FROM FETAX TEST 1
(JP-4#1--JP-4 AND AGAROSE)

JP-4 Concentrations (%)	% Malformation	% Mortality
0 (Control)	11.1	10.0
0.125	15.0	0.0
1.25	10.0	3.0
3.125	53.3	18.0
6.25	57.9	53.0
12.5	100.0	98.0

TABLE 11. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2
(JP-4#2--JP-4 AND AGAROSE WITH MICROSOMES)

Concentrations (% JP-4)	Controls				1.0		1.25		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe									1	5		1	5	6	1	1		2
Stunted																		
Gut	2	1	1		2		2	2	8	10	6	9	7	10	1	1		
Edema																		
Multiple																		
Cardiac									4			2		3				
Abdominal									1	3								
Facial							1			1								
Cephalic																		
Optic										1		1						
Tail									1									
Notochord									1		2							
Fin																		
Face	1	1			1				2									
Eye	1		1		1		1		1		1	1		4	2	4	2	
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	3	2	2		2	0	2	2	9	14	7	10	11	16	3	2	0	2
No. Scored	18	20	20	20	19	19	20	19	20	18	19	20	12	16	19	18	0	2

TABLE 12. MALFORMATION CHARATERIZATION AND QUANTIFICATION OF TEST 3
(JP-4#3--JP-4 AND AGAROSE WITHOUT MICROSOMES)

Concentrations (% JP-4)	Controls				1.25		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2
Severe							1	1	1		7	1	14	15		
Stunted																
Gut		1			1	5	5	4	3			7	2			
Edema																
Multiple																
Cardiac									1							
Abdominal							1									
Facial												1				
Cephalic																
Optic						1										
Tail			1									1				
Notochord				1	1	2	2	4				4	1			
Fin																
Face					1		1					2				
Eye						1	2	1				8				
Brain																
Hemorrage																
Cardiac																
Blisters									1							
Other																
No. Malformed	0	1	1	1	3	5	8	9	4	0	7	14	16	15	0	0
No. Scored	7	19	15	9	18	16	19	16	13	0	7	17	16	15	0	0

TABLE 13. MORTALITY DATA FOR FETAX TEST 2 (JP-4#2--JP-4 AND AGAROSE WITH MICROSOMES)

JP-4 (%)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	2	0	10.0	0.0	
Control	0	0	0	0	0	0	0	0	0.0	0.0	3.0
1.0	0	1	0	0	0	0	1	0	5.0	5.0	5.0
1.25	0	0	0	0	0	0	0	1	0.0	5.0	2.5
3.75	0	2	0	0	0	0	0	0	0.0	10.0	5.0
5.0	1	0	0	0	0	0	0	0	5.0	0.0	2.5
6.25	1	0	0	0	0	1	7	3	40.0	20.0	30.0
7.5	0	0	0	0	0	0	1	2	5.0	10.0	8.0
10.0	0	0	0	9	0	0	20	9	100.0	90.0	95.0

TABLE 14. MORTALITY DATA FOR FETAX TEST 3 (JP-4#3--JP-4 AND AGAROSE WITHOUT MICROSOMES)

JP-4 (%)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	13	1	65.0	5.0	
Control	0	0	0	0	0	0	5	11	25.0	55.0	38.0
1.25	1	1	0	0	1	2	0	1	10.0	20.0	15.0
3.75	0	0	0	0	1	0	0	4	5.0	20.0	13.0
5.0	3	2	2	18	1	0	1	0	35.0	100.0	68.0
6.25	2	0	6	0	0	0	5	3	65.0	15.0	40.0
7.5	1	3	0	0	0	0	3	2	20.0	25.0	23.0
10.0	5	2	1	3	1	0	13	15	100.0	100.0	100.0

TABLE 15. RESULTS FROM FETAX TEST 2 (JP-4#2--JP-4 + MICROSOMES). EXPOSURE METHOD USED WAS JP-4 IN AGAROSE

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	3.0	9.0	9.5
1.00	5.0	5.3	9.7
1.25	3.0	10.3	9.3
3.75	5.0	60.5	8.5
5.00	3.0	43.6	8.6
6.25	30.0	95.4	7.7
7.5	8.0	13.5	9.0
10.0	95.0	100.0	6.9

TABLE 16. RESULTS FROM FETAX TEST JP-4#4, JP-4 WITHOUT MICROSOMES. EXPOSURE METHOD USED WAS JP-4 IN AGAROSE

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	38.0	6.0	9.1
1.25	15.0	26.5	8.8
3.75	13.0	48.6	8.5
5.0	68.0	30.8	7.9
6.25	40.0	87.5	7.9
7.5	23.0	100.0	7.3
10.0	100.0		

TABLE 17. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST JP-4#4, JP-4 AND AGAROSE WITHOUT MICROSOMES

Concentrations (% JP-4)	Controls				1.25		2.5		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe				2	2	4	6	4	3	8	5	7	10	17	11	7		
Stunted																		
Gut	2	1		1	6	6	9	8	3	6	8	5	8	1	4	7		
Edema																		
Multiple																		
Cardiac					1	2		1		1			2		1			
Abdominal					2	2		1										
Facial													3		1	2		
Cephalic																		
Optic					2	2		2							2	1		
Tail								1										
Notochord					7	5	6	1		3						2		
Fin												1						
Face					3	5	6			2	1	1	2					
Eye							7		1	2	2	1	2					
Brain													1					
Hemorrage																		
Cardiac						1	2			1								
Blisters																		
Other																		
No. Malformed	2	1	0	3	11	13	18	13	7	14	12	14	17	17	19	14	0	0
No. Scored	14	16	11	18	12	20	18	18	20	14	19	20	19	17	20	20	0	0

TABLE 18. MORTALITY DATA FOR FETAX TEST JP-4#4, JP-4 AND AGAROSE WITHOUT MICROSOMES

JP-4 (%)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	6	4	30.0	20.0	
Control	0	0	0	0	0	0	9	2	45.0	10.0	26.0
1.25	0	0	7	0	1	0	0	0	40.0	0.0	20.0
2.5	0	0	0	0	1	1	1	1	10.0	10.0	10.0
3.75	0	0	0	6	0	0	0	0	0.0.	30.0	15.0
5.0	0	0	0	0	1	0	0	0	5.0	0.0	3.0
6.25	0	0	0	0	1	1	0	2	5.0	15.0	10.0
7.5	0	0	0	0	0	0	0	0	0.0	0.0	0.0
10.0	0	0	4	0	16	20	0	0	100.0	100.0	100.0

TABLE 19. RESULTS FROM FETAX TEST JP-4#4
(JP-4 EXPOSURE WITHOUT MICROSOMES)

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	26.0	10.2	9.3
1.25	20.0	75.0	8.9
2.5	10.0	86.1	8.9
3.75	15.0	61.8	8.8
5.0	3.0	69.2	8.8
6.25	10.0	94.4	8.1
7.5	0.0	82.5	9.0
10.0	100.0		

TABLE 20. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST JP-4#5, JP-4 AND AGAROSE WITH MICROSOMES

Concentrations (% JP-4)	Controls				1.25		2.5		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe	1				1		1			2	3		9	2	17	1	7	2
Stunted		1	4	2	10	6	10	14	14	11	8	6	9	12		4		10
Gut																		
Edema																		
Multiple			1															
Cardiac			1		1					1				1				
Abdominal			1			1												
Facial									3	2				1				
Cephalic																		1
Optic									1					1				
Tail								2	1					2				
Notochord																		
Fin					2		1		3	1		1	1	2				1
Face																		
Eye																		
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	1	1	4	2	10	6	13	14	14	13	11	6	18	15	17	5	7	16
No. Scored	20	18	19	19	20	20	19	19	17	17	20	19	18	20	17	19	7	18

TABLE 21. DAILY MORTALITY DATA FOR TEST JP-4#5, JP-4 AND AGAROSE WITH MICROSOMES

JP-4 (%)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	0	2	0.0	10.0	5.0
Control	0	0	0	0	0	0	1	1	5.0	5.0	0.0
1.25	0	0	0	0	0	0	0	0	0.0	0.0	5.0
2.5	0	0	0	0	1	1	0	0	5.0	5.0	15.0
3.75	0	0	3	0	0	3	0	0	15.0	15.0	2.5
5.0	0	0	0	0	0	1	0	0	0.0	5.0	5.0
6.25	0	0	0	0	2	0	0	0	10.0	0.0	10.0
7.5	0	0	0	0	1	0	2	1	15.0	5.0	38.0
10.0	0	0	0	0	13	2	0	0	65.0	10.0	

TABLE 22. RESULTS FROM FETAX TEST JP-4#5
(JP-4 AND AGAROSE WITH MICROSOMES)

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	5.0	10.5	10.0
1.25	0.0	40.0	9.2
2.5	5.0	71.1	8.9
3.75	15.0	79.4	8.3
5.0	3.0	43.6	8.8
6.25	5.0	86.8	8.2
7.5	10.0	61.1	8.3
10.0	38.0	92.0	7.5

TABLE 23. MALFORMATION CHARACTERIZATION AND QUANTIFICATION OF FETAX TEST JP-4#6, JP-4 AND AGAROSE WITHOUT MICROSOMES

Concentrations (% JP-4)	Controls				1.25		2.5		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe							2				19	18	11	16	12	20		19
Stunted				2	4	1	6	5	19	9								
Gut																		
Edema																		
Multiple																		
Cardiac					1		1	1										
Abdominal					1		1	1										
Facial																		
Cephalic																		
Optic																		
Tail							4		19	2								
Notochord																		
Fin				1	4		6	2	19	9								
Face					4		8	2	19	8								
Eye																		
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	0	0	0	2	5	1	11	5	19	9	19	18	11	16	12	20	0	19
No. Scored	20	18	20	15	8	20	20	19	19	9	19	18	11	16	12	20	0	19

TABLE 24. DAILY MORTALITY DATA FOR FETAX TEST JP-4#6, JP-4 AND AGAROSE WITHOUT MICROSOMES

JP-4 (%)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	0	2	0.0	10.0	9.0
Control	0	0	0	0	0	0	0	5	0.0	25.0	30.0
1.25	0	0	1	0	3	0	8	0	60.0	0.0	2.5
2.5	0	0	0	0	0	1	0	0	0.0	5.0	30.0
3.75	0	0	0	0	0	6	1	5	5.0	55.0	8.0
5.0	0	0	0	1	0	0	1	1	5.0	10.0	33.0
6.25	0	0	0	0	0	0	9	4	45.0	20.0	20.0
7.5	0	0	0	0	2	0	6	0	40.0	0.0	53.0
10.0	0	0	2	0	1	0	17	1	100.0	5.0	

TABLE 25. RESULTS FROM FETAX TEST JP-4#6
(JP-4 AND AGAROSE WITHOUT MICROSOMES)

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	9.0	2.7	9.0
1.25	30.0	21.4	8.9
2.5	3.0	41.0	8.8
3.75	30.0	100.0	7.7
5.0	8.0	100.0	8.1
6.25	33.0	100.0	7.0
7.5	20.0	100.0	8.0
10.0	53.0	100.0	8.6

TABLE 26. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR FETAX TEST JP-4#7, JP-4 AND AGAROSE WITH MICROSOMES

Concentrations (% JP-4)	Controls				1.25		2.5		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe		1	1	2	1	1				3			2			20	13	
Stunted																		
Gut	2		2		2	2	4	2	7	12	20	18	1	19	15			17
Edema																		
Multiple																		2
Cardiac									1									
Abdominal								1										
Facial																		
Cephalic																		
Optic																		
Tail																		
Notochord										2	8	6		1	3			
Fin																		
Face	1		1					2	1	7	20	18	1	19				
Eye					1			1	1	1	20	17		19	15			2
Brain			1															
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	2	1	4	2	3	3	4	3	7	15	20	18	3	19	15	20	13	17
No. Scored	20	20	20	19	20	18	19	18	18	18	20	18	3	19	15	20	13	20

TABLE 27. DAILY MORTALITY DATA FOR FETAX TEST JP-4#7, JP-4 AND AGAROSE WITH MICROSOMES

Concentration (% JP-4)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	0	0	0.0	0.0	
Control	0	0	0	0	0	0	0	1	0.0	5.0	1.0
1.25	0	2	0	0	0	0	0	0	0.0	10.0	5.0
2.5	0	0	0	2	0	0	1	0	5.0	10.0	8.0
3.75	1	0	0	0	0	0	1	2	10.0	10.0	10.0
5.0	0	0	0	0	0	0	0	2	0.0	10.0	5.0
6.25	0	0	1	0	1	1	15	0	85.0	5.0	45.0
7.5	0	0	0	0	1	0	4	0	25.0	0.0	13.0
10.0	0	0	0	0	1	0	6	0	35.0	0.0	18.0

TABLE 28. RESULTS FROM FETAX TEST JP-4#7
(JP-4 AND AGAROSE WITH MICROSOMES)

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	1.0	11.4	9.4
1.25	5.0	15.8	9.1
2.5	8.0	18.9	9.2
3.75	10.0	61.1	8.7
5.0	5.0	100.0	7.7
6.25	45.0	100.0	7.7
7.5	13.0	100.0	6.9
10.0	18.0	90.9	8.1

TABLE 29. SUMMARY OF THE LC50, EC50 AND TI VALUES FOR PRELIMINARY FETAX TESTS USING JP-4 AND AGAROSE (PERFORMED WITH AND WITHOUT MICROSOMES)

Test #	LC50 (mortality) (% JP-4)	EC50 (malformation) (% JP-4)	TI
JP-4#1 without MAS	8.4	3.5	2.4
JP-4#2 without MAS	7.716	3.032	3.3
JP-4#3 with MAS	7.699	2.320	2.5
JP-4#4 without MAS	8.779	1.967	4.4
JP-4#5 with MAS	10.307	1.771	5.8
JP-4#6 without MAS	9.432	2.250	4.2
JP-4#7 with MAS	12.32	1.907	4.7

TABLE 30. SUMMARY OF DATA FROM WEATHERED JP-4 VS. JP-4 TEST (WJP-4#1)

Concentration of JP-4 (% JP-4)	Mortality (%)		Malformation (%)	
	JP-4	weathered JP-4	JP-4	weathered JP-4
Controls	1.25	1.25	3.8	3.8
0.5	22.5*	5.0	100	100
0.8	2.5	0.0	100	100
1.25	10.0	2.5	100	100
2.5	0.0	12.5	100	100
3.75	100	100		
5.0	100	100		
6.25	100	100		
7.5	100	100		

* Some of this mortality was due to crowding effects which occurred when some embryos were stuck in a depression in the agarose.

TABLE 31. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR FETAX TEST JCA#1, JP-4, CORN OIL AND AGAROSE

Concentrations (% JP-4)	Controls				1.25		2.5		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe		1				5	7	5	16	9	12	8	1	8	9	7	5	1
Stunted																		
Gut	3	1	3	1	9													
Edema																		
Multiple																		
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic																		
Tail																		
Notochord					19													
Fin																		
Face	2		1															
Eye					7													
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	3	2	4	1	19	8	7	5	1	9	12	8	1	8	9	7	5	1
No. Scored	8	19	17	15	19	8	7	5	16	9	12	8	1	8	9	7	5	1

TABLE 32. SUMMARY OF RESULTS FOR FETAX TEST JCA#1 (JP-4, CORN OIL, AND AGAROSE)

JP-4 (%)	% Mortality	% Malformation
Controls	26	16.9
1.25	33	100
2.5	70	100
3.75	38	100
5.0	50	100
6.25	78	100
7.5	60	100
10.0	85	100

TABLE 33. RESULTS FROM FETAX TEST JCA#2 (JP-4, CORN OIL, AND AGAROSE)

% JP-4	% Mortality	% Malformation	Mean Growth (mm)
Controls	18	16.7	9.1
1.25	33	100	7.8
2.5	15	100	7.9
3.75	55	100	7.0
5.0	93	100	6.0
6.25	100		
7.5	100		
10.0	100		

TABLE 34. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 3 (JCA#3), JP-4, CORN OIL AND AGAROSE

Concentrations (% JP-4)	Controls				1.25		2.5		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe			1	2	8	17	9	5	8	5	4		1		4	1		
Stunted																		
Gut		1																
Edema																		
Multiple																		
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic																		
Tail																		
Notochord																		
Fin																		
Face	1	3	1															
Eye	1	1	1	1														
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	3	3	2	3	8	17	9	5	8	5	4	0	1	0	4	1	0	0
No. Scored	17	18	12	19	8	17	9	5	8	5	4	0	1	0	4	1	0	0

TABLE 35. DAILY MORTALITY DATA FOR TEST 3 (JCA#3), JP-4, CORN OIL AND AGAROSE

Concentration (% JP-4)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	3	2	15.0	10.0	
Control	0	0	0	0	0	0	8	1	40.0	5.0	18.0
1.25	1	0	0	1	2	0	9	2	60.0	15.0	38
2.5	0	0	0	0	2	4	9	11	55.0	75.0	65.0
3.75	0	1	0	4	3	3	9	7	60.0	75.0	68
5.0	0	0	0	9	5	3	11	8	80.0	100.0	90.0
6.25	0	0	0	14	5	3	14	3	95.0	100.0	98
7.5	0	0	0	8	1	3	15	8	80.0	95.0	88
10.0	0	0	0	0	0	1	20	19	100.0	100.0	100.0

TABLE 36. RESULTS FROM FETAX TEST 3, (JAC#3) JP-4, CORN OIL AND AGAROSE.

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	18	16.7	9.1
1.25	38	100	7.2
2.5	65	100	6.2
3.75	68	100	6.2
5.0	90	100	6.5
6.25	98	100	7.9
7.5	88	100	7.2
10.0	100		

TABLE 37. DAILY MORTALITY DATA FOR TEST 4 (JCA#4) JP-4 AND AGAROSE

Concentration (% JP-4)	24 hours		48 hours		72 hours		96 hours		% Mortality per replicate		Average % Mortality
Replicate	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	1,3	2,4	
Control	0	0	0	0	0	0	3	2	15	10	
Control	0	0	0	0	0	0	8	1	40	5	18
1.25	1	0	0	0	4	0	6	2	55	10	33
2.5	1	0	0	0	0	0	2	3	15	15	15
3.75	3	0	0	0	3	3	5	8	55	55	55
5.0	0	0	2	0	4	1	14	16	100	85	93
6.25	0	1	0	0	5	3	15	16	100	100	100
7.5	0	0	1	0	6	8	13	12	100	100	100
10.0	0	0	1	16	4	6	15	0	100	100	100

TABLE 38. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 4 (JCA#4) JP-4 AND AGAROSE

Concentrations (% JP-4)	Controls				1.25		2.5		3.75		5.0		6.25		7.5		10.0	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe			1	2	9	18	17	17	9	9		3						
Stunted																		
Gut		1																
Edema																		
Multiple																		
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic																		
Tail																		
Notochord																		
Fin																		
Face	1	3	1															
Eye	1	1	1	1														
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	3	3	2	3	9	18	17	17	9	9	0	3	0	0	0	0	0	0
No. Scored	17	18	12	19	9	18	17	17	9	9	0	3	0	0	0	0	0	0

TABLE 39. RESULTS FROM FETAX TEST 4 (JCA#4) , JP-4 AND AGAROSE

JP-4 Concentration (%)	% Mortality	% Malformation	Mean Growth (mm)
Controls	18	16.7	9.1
1.25	33	100	7.8
2.5	15	100	7.9
3.75	55	100	7.0
5.0	93	100	6.0
6.25	100		
7.5	100		
10.0	100		

TABLE 40. COMPARISON OF % MORTALITY FOR VOLUMES OF JP-4 AND SCF

Test Sample	Volume (µl)	Mortality (%)
JP-4[1]	40	14
SCF[2]	60	100
JP-4[3]	300	100

[1] The lowest volume of JP-4 that has been tested
[2] 100% mortality occurred at 48 hours
[3] The lowest volume of JP-4 which induced 100% mortaltiy. 100% mortality occurred at 96 hours.

The LC50 of JP-4 is approximately 3.3%

TABLE 41. SUMMARY OF RESULTS FROM SCF FETAX TEST 2 (SCF#2)

Treatment	% Mortality	% Malformation
Control	4	3.8
B	100	
G	67.5	100
O	100	
S	100	

TABLE 42. EXPERIMENTAL DESIGN TO TEST THE TOXICITY OF THE SCFE METHOD (TEST SAND#1)

Dish	Agarose (mL)	Corn Oil (mL)	FETAX solution (mL)
FETAX Controls	0	0	8
Control	7	0	1
Control	7	1	0
Control	7	0.125	0.875
Corn Oil	7	0.25	0.75
Corn Oil	7	0.5	0.5
SCFE	7	0.125	0.875
SCFE	7	0.25	0.75
SCFE	7	0.5	0.5

TABLE 43. RESULTS FROM TEST SAND#2, PERFORMED TO ASSESS THE TOXICITY OF BLASTING SAND AND SILICA

Exposure	% Mortality	% Malformation
Controls	4.0	7.22
Control Jars	5.0	12.28
Blasting Sand	5.0	13.15
Silica	16.66	11.00

TABLE 44. RESULTS FROM AQUEOUS EXTRACTION TESTS #1 AND #2 (AE#1 AND AE#2)

Test Sample	Aqueous Extraction Test #1		Aqueous Extraction Test #2	
	% Mortality	% Malformation	% Mortality	% Malformation
Control	71.0	34.0	5.0	11.5
80EA6	64.0	30.0	14.0	9.0
80EA7	65.0	40.0	4.0	16.7
80KC5	71.0	32.0	8.0	4.0

TABLE 45. SUMMARY OF RESULTS FROM AQUEOUS EXTRACTION TESTS 3 AND 4 (AE#3 AND AE#4)

Sample ID	Mortality (%)		Malformation (%)	
	Test 3	Test 4	Test 3	Test 4
Control	2.0	5.0	9.0	4.0
K	2.0		0.0	
B	10.0	13.0	5.0	8.0
O	6.0	3.0	4.0	7.0
G	12.0	10.0	0.0	0.0
S	8.0	7.0	6.0	4.0
N	20.0	47.0	16.5	13.0
E	16.0	20.0	8.0	4.0
R	14.0	3.0	5.0	7.0
F	16.0	3.0	5.0	10.0
C	8.0	7.0	0.0	0.0

TABLE 46. MORTALITY AND MALFORMATION RESULTS FROM PRELIMINARY DIRECT EXPOSURE TEST #1 (PDE#1)

Sample	% Mortality	% Malformation
Control	7.0	9.0
80EA6	100.0	
80EA7	3.0	16.0
80KC5	6.0	12.0

TABLE 47. SUMMARY OF PERCENT MORTALITY AND PERCENT MALFORMATION FOR PRELIMINARY DIRECT EXPOSURE TESTS 2 AND 3 (PDE#2, AND PDE#3)

Test Sample	Mortality (%)		Malformation (%)	
	PDE#2	PDE#3	PDE#2	PDE#3
Control	58.0	25.0	14.0	17.0
K	76.0	25.0	8.0	7.0
B	100.0	100.0		
O	100.0	100.0		
G	100.0	75.0		30.0
S	100.0	100.0		
N	100.0	100.0		
E	100.0	100.0		
R	100.0	92.5		100.0
F	100.0	100.0		
C	100.0	100.0		

TABLE 48. SUMMARY OF PRELIMINARY DIRECT EXPOSURE TEST 4 (PDE#4) USING SOIL FROM K SITE SPIKED WITH JP-4

Treatment	% Mortality	% Malformation
Control	0	5
Not spiked K (5 mL soil/vessel)	3	3
Spiked K (5 mL soil/vessel)	7	100
Spiked K (%) mL soil/vessel)	100	

TABLE 49. SUMMARY OF COLLECTION SITES AND THE TREATMENT AREA FROM WHERE THE SAMPLES WERE COLLECTED

Time	Treatment					
	GZ	NC	NCC	CC	CCC	K-CON
Pre Remediation	S	O	E	R	F	K
	N	B		C		
				G		
During Remediation	ZG	W	Y	ZB		KC
		X		ZC		
		Z		ZD		
		ZA		ZE		
				ZF		
Post Remediation	ZGA	ZM	ZP	ZT	ZU	KD
	ZL	ZK	ZQ	ZX	ZW	
		ZN	ZR	ZY	ZV	
		ZO		ZZ		
		ZS		ZZA		

TABLE 50. SUMMARY OF THE RESULTS FROM FETAX TEST 1 OF THE PRE REMEDIATION SOIL SAMPLES (TEST PRE-R#1)

Sample ID	% Mortality	% Malformation	Mean Length (cm)
CONTROLS	20	7	0.89
K	21	0	0.91
B	55	11	0.83
O	70	0	0.90
G	8	0	0.86
S	100		
N	60	44	0.85
E	3	8	0.84
R	28	7	0.91
F	33	11	0.87
C	55	0	0.85

TABLE 51. SUMMARY OF THE RESULTS FROM FETAX TEST 2 OF THE PRE REMEDIATION SOIL SAMPLES (TEST PRE-R#2)

Sample ID	% Mortality	% Malformation	Mean Length (cm)
CONTROLS	3	17	0.85
K	2	10	0.86
B	100		
O	27	68	0.80
G	0	12	0.82
S	47	100	0.58
N	100		
E	100		
R	20	13	0.85
F	72	100	0.65
C	70	100	0.62

TABLE 52. SUMMARY OF THE RESULTS FROM FETAX TEST 3 OF THE PRE REMEDIATION SOIL SAMPLES (TEST PRE-R#3)

Sample ID	% Mortality	% Malformation	Mean Length (cm)
CONTROLS	2	6	0.88
K	3	8	0.89
B	78	100	0.65
O	12	50	0.77
G	2	6	0.87
S	100		
N	100		
E	100		
R	5	4	0.86
F	55	90	0.79
C	62	100	0.74

TABLE 53. SUMMARY OF MALFORMATION AND MORTALITY DATA FOR PRE REMEDIATION SOIL SAMPLES

Treatment	% Mortality			% Malformation		
	PRE-R#1	PRE-R#2	PRE-R#3	PRE-R#1	PRE-R#2	PRE-R#3
GZ	80	73.5	100	44	100	
NC	42.7	75.7	63.3	6.3	68	75
CC	31	54	31	4.5	56.25	50
K-CON	21	2	3	0	10	8

TABLE 54. TESTING SCHEME FOR THE DURING REMEDIATION SOIL SAMPLES

Highest soil depth	Lowest soil depth	High middle	Low middle
D-R#1	D-R#2	D-R#3	D-R#4
KC 1 &2	KC 3&4	KC 1 &2	KC 3& 4
W8	W11	W9	W10
X11	X3	X1	X2
Z12	Z3	Z1	Z2
ZA1	ZA4	ZA2	ZA3
ZB8	ZB2	ZB9	ZB1
ZC10	ZC2	ZC11	ZC1
ZE1	ZE4	ZE2	ZE3
ZF1	ZF4	ZF2	ZF3
BLASTING SAND		Y 11&10	Y 1 & 2
		ZD 1 & 2	ZD 3 & 4
		ZG 10 & 9	ZG 1 & 12

TABLE 55. SUMMARY OF THE RESULTS FROM FETAX DURING REMEDIATION TEST 1 (D-R#1)

Sample	% Mortality	% Malformation	Mean Length (cm)
Controls	8.3	7.3	0.97
KC 1 &2	35.0	10.3	0.98
W8	100		
X11	100		
Z12	40.0	8.13	0.97
ZA1	71.7	7.69	0.93
ZB8	50.0	13.33	0.97
ZC10	90.0	100	0.84
ZE1	78.3	0	0.97
ZF1	35.0	17.14	0.97
BLASTING SAND	56.7	50.0	0.95

TABLE 56. SUMMARY OF DIRECT EXPOSURE FETAX TEST 2 DURING REMEDIATION (D-R#2)

Sample	% Mortality	% Malformation	Mean Length (mm)
Controls	3.3	8.62	9.47
KC 3&4	5	15.74	9.34
W11	100		
X3	100		
Z3	100		
ZA4	13.3	18.03	9.58
ZB2	35	23.8	9.28
ZC2	31.7	6	9.53
ZE4	13.3	21.15	9.43
ZF4	3.3	15.48	9.21
Blasting Sand	16.7	26.62	9.08

TABLE 57. SUMMARY OF THE RESULTS FROM DIRECT EXPOSURE TEST 3 ON THE DURING REMEDIATION SAMPLES (D-R#3)

Sample	% Mortality	% Malformation	Mean Growth (mm)
Controls	5.0	3.51	9.80
KC 1,2	16.7	4	9.72
W9	100		
X1	100		
Z1	100		
ZA2	100		
ZB9	10	5.56	9.62
ZC11	100		
ZE2	8.3	7.27	9.60
ZF2	11.7	11.32	9.95
ZD1 & 2	100		
ZG10,11	100		
Y 11,10	8.3	23.64	9.67

TABLE 58. SUMMARY OF THE RESULTS FROM DIRECT EXPOSURE TEST 4 ON THE DURING REMEDIATION SAMPLES (D-R#4)

Sample	% Mortality	% Malformation	Mean Growth (mm)
Controls	6.7	3.57	9.92
KC 3,4	15	7.84	9.67
W10	100		
X2	100		
Z2	100		
ZA3	100		
ZB1	100		
ZC1	33.3	17.50	9.33
ZE3	13.3	11.54	9.47
ZF 3	23.3	17.39	9.63
Y 1,2	23.3	8.70	9.62
ZD 3,4	33.3	12.5	9.74
ZG 1,2	100		
ZG8	98.3	100	9.9

TABLE 59. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYER 1 OF POST REMEDIATION SAMPLES (POST-R#1)

Samples	FETAX Controls				SAND		KD		ZS		ZT		ZX		ZZA	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2
Severe											1					
Stunted																
Gut			1		1		2	2				1		2		
Edema												1		3		
Multiple																
Cardiac																
Abdominal																
Facial																
Cephalic																
Optic																
Tail								1								
Notochord								1		1	1					
Fin																
Face		1				1	1	2	1	2	1	1	2	2	1	1
Eye																
Brain								1								
Hemorrage								1	4	2	1	3	4	4		
Cardiac																
Blisters																
Other																
No. Malformed	0	1	1	0	1	1	3	4	5	4	4	4	5	6	1	1
No. Scored	30	30	29	29	30	30	30	30	30	25	30	30	30	27	27	13

TABLE 60. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYER 2 OF POST REMEDIATION SAMPLES (POST-R#2)

Samples	FETAX controls				SAND		KD		ZS		ZT		ZX		ZY		ZZA	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																		
Stunted																		
Gut				2	2	1	2	1		3	1	1		1	3	1		
Edema																		
Multiple															1			
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic																		
Tail							1				1							
Notochord							1	1	1		1				11	2		
Fin																		
Face		1	1	1		1	1	1	3	3	1	4	1	1	2	2	2	1
Eye																		
Brain												1						
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	0	1	1	3	2	2	3	1	4	4	2	4	1	1	11	2	2	1
No. Scored	29	30	29	30	30	30	30	29	28	28	27	24	30	27	11	2	28	25

TABLE 61. SUMMARY OF RESULTS FROM TEST 1 LAYER 1 OF POST REMEDIATION SOIL SAMPLES (POST-R#1)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	1.67	1.70	0.96
Sand Controls	0.0	3.33	0.95
KD	0.0	11.67	0.95
ZN	100.0		
ZO	100.0		
ZS	8.33	16.33	0.89
ZT	0.0	13.33	0.88
ZU	100.0		
ZV	100.0		
ZW	100.0		
ZX	5.0	19.44	0.93
ZY	100.0		0.86
ZZA	33.3	5.70	0.95

TABLE 62. SUMMARY OF RESULTS FROM TEST 2 LAYER 1 OF POST REMEDIATION SOIL SAMPLES (POST-R#2)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	1.67	4.20	0.86
Sand Controls	0.0	6.67	0.89
KD	1.67	8.45	0.88
ZN	100.0		
ZO	100.0		
ZS	6.67	14.29	0.85
ZT	15.0	12.04	0.86
ZU	100.0		
ZV	100.0		
ZW	100.0		
ZX	5.0	3.52	0.86
ZY	78.33	100.0	0.86
ZZA	11.67	5.57	0.90

TABLE 63. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 1 LAYER 1 (POST-R#1)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	9.0	7.3	8.2	7.0	7.0	6.7	7.2	6.8
Sand Controls	8.6	7.2	7.8	7.0	6.9	6.9	7.4	6.8
KD	8.5	7.1	7.6	7.1	7.1	6.9	7.5	6.9
ZN	8.3	7.1	7.5	7.1	7.1	6.9	7.6	7.0
ZO	8.2	7.1	7.4	7.1	7.3	6.9	7.5	7.1
ZS	8.1	6.9	7.2	7.0	7.3	6.9	7.5	7.0
ZT	8.2	6.9	7.3	7.0	7.1	7.0	7.4	6.9
ZU	8.1	7.4	7.3	6.9	7.5	7.3	7.1	6.9
ZV	8.1	7.4	7.3	6.9	7.5	7.2	7.3	6.8
ZW	8.1	7.3	7.4	7.0	7.5	7.1	7.4	7.1
ZX	8.1	7.3	7.5	7.1	7.6	7.1	7.5	7.1
ZY	8.1	7.2	7.5	7.1	7.7	7.1	7.5	7.1
ZZA	8.0	7.2	7.5	7.1	7.7	7.1	7.6	7.0

TABLE 64. SUMMARY OF PH VALUES TAKEN AT END OF TEST 2 LAYER 1 (POST-R#2)

Sample	pH value at end of 96 hours
FETAX Controls	7.06
Sand Controls	7.18
KD	7.16
ZN	6.91
ZO	6.76
ZS	7.19
ZT	7.12
ZU	6.73
ZV	6.67
ZW	6.78
ZX	7.11
ZY	6.91
ZZA	7.20

TABLE 65. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYER 2 OF POST REMEDIATION SAMPLES (POST-R#3)

Samples	FETAX Controls				SAND		KD		ZK		ZL		ZN		ZR		ZT		ZY		ZZ		ZZA	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																								
Stunted																	1							
Gut			1		1		1	1	7	2	2	1				1	2	2		3	2	1	2	2
Edema																								
Multiple											1									2		1		
Cardiac																								
Abdominal																								
Facial																								
Cephalic																								
Optic																								
Tail				1						1														
Notochord				1						1										3				
Fin				1																3				
Face		1	1		1	1	2	1	8	3	3	2	1		2		3	4		2	1	3	1	2
Eye									5	1								1		1		1		1
Brain																								
Hemorrage																								
Cardiac																								
Blisters																								
Other																								
No. Malformed	0	1	2	1	2	1	3	1	8	4	3	3	1	0	2	1	3	4	0	3	2	3	3	3
No. Scored	30	25	30	30	21	20	30	7	25	27	21	21	2	0	25	25	28	21	0	3	25	28	23	26

TABLE 66. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2 LAYER 2 OF POST REMEDIATION SAMPLES (POST-R#4)

Samples	FETAX Control				SAND		KD		ZK		ZL		ZN		ZO		ZP		ZR		ZT		ZZ		ZZA	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																										
Stunted					1	1																				
Gut								1	1	6	4	2			3	4		5		3	3	9	3	1	3	4
Edema																										
Multiple									1	2						1										2
Cardiac															1											
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail										2																
Notochord										2		1								1						
Fin												1								1						
Face	1	3		2	1			1	2	5	5	3	1	2	3	4		5	1	2	3	8	6	1	3	3
Eye					1	1				3	1				1	1		1				5	4	1	3	2
Brain																							4	1	2	2
Hemorrage																										
Cardiac																										
Blisters																										
Other																										
No. Malformed	1	3	0	2	2	2	0	2	2	7	7	5	1	2	4	4	0	5	1	3	4	9	6	1	3	5
No. Scored	30	30	30	30	17	22	3	26	28	28	25	27	1	2	4	4	0	5	24	27	15	9	25	11	30	30

TABLE 67. SUMMARY OF PH VALUES TAKEN AT END OF TEST 1 LAYER 2 (POST-R#3)

Sample	pH value at end of 96 hours
FETAX Controls	7.4
Sand Controls	7.5
KD	7.2
ZK	7.4
ZL	7.7
ZM	*
ZN	6.9
ZO	7.2
ZP	6.8
ZQ	*
ZR	7.4
ZT	7.5
ZY	7.2
ZZ	7.3
ZZA	7.6

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 68. SUMMARY OF PH VALUES TAKEN AT END OF TEST 2 LAYER 2 (POST-R#4)

Sample	pH value at end of 96 hours
FETAX Controls	7.2
Sand Controls	7.3
KD	6.9
ZK	7.0
ZL	7.1
ZM	*
ZN	6.7
ZO	6.7
ZP	6.9
ZQ	*
ZR	6.9
ZT	6.9
ZY	*
ZZ	7.5
ZZA	7.4

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 69. SUMMARY OF RESULTS FROM TEST 1 LAYER 2 OF POST REMEDIATION SOIL SAMPLES (POST-R#3)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	4.17	3.50	0.97
Sand Controls	31.67	7.26	0.94
KD	38.33	10.81	0.95
ZK	13.33	23.08	0.89
ZL	30.00	14.29	0.88
ZM	100.0		
ZN	69.67	50.0	0.90
ZO	100.0		
ZP	100.0		
ZQ	100.0		
ZR	15.0	5.88	0.97
ZT	18.33	14.29	0.91
ZY	95.0	100.0	0.72
ZZ	11.67	8.43	0.93
ZZA	18.33	12.24	0.96

TABLE 70. SUMMARY OF RESULTS FROM TEST 2 LAYER 2 OF POST REMEDIATION SOIL SAMPLES (POST-R#4)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	0.0	5.0	0.97
Sand Controls	35.0	10.43	0.96
KD	51.67	6.9	0.97
ZK	6.67	16.07	0.86
ZL	13.33	23.08	0.89
ZM	100.0		
ZN	95.0	100.0	0.73
ZO	86.67	100.0	0.78
ZP	91.67	100.0	0.70
ZQ	100.0		
ZR	15.0	7.84	0.92
ZT	60.0	54.17	0.83
ZY	100.0		
ZZ	40.0	19.44	0.95
ZZA	0.0	13.33	0.94

TABLE 71. SUMMARY OF RESULTS FROM TEST 1 LAYER 3 OF POST REMEDIATION SOIL SAMPLES (POST-R#5)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	2.50	1.73	0.98
Sand Controls	8.33	1.72	0.98
KD	28.33	6.98	0.95
ZK	13.33	13.46	0.94
ZL	6.67	44.64	0.80
ZM	100.0		
ZO	100.0		
ZP	5.0	10.53	0.92
ZQ	38.33	13.51	0.90
ZR	10.0	8.26	0.91
ZS	13.33	17.31	0.92
ZT	100.0		
ZU	71.67	17.65	0.96
ZV	13.33	17.31	0.91
ZW	3.33	15.52	0.93
ZX	0.0	10.0	0.96
ZZ	8.33	12.73	0.94
ZZA	41.67	8.57	0.94

TABLE 72. SUMMARY OF RESULTS FROM TEST 2 LAYER 3 OF POST REMEDIATION SOIL SAMPLES (POST-R#6)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	1.67	2.50	0.94
Sand Controls	8.33	9.33	0.92
KD	15.0	11.76	0.89
ZK	13.33	13.46	0.86
ZL	100.0		
ZM	100.0		
ZO	100.0		
ZP	26.67	25.0	0.81
ZQ	30.0	47.62	0.79
ZR	11.67	13.21	0.80
ZS	33.33	15.0	0.80
ZT	93.33	100.0	0.66
ZU	30.0	9.52	0.86
ZV	36.67	15.79	0.86
ZW	58.33	16.0	0.87
ZX	5.00	10.53	0.86
ZZ	23.33	8.70	0.87
ZZA	28.33	9.30	0.88

TABLE 73. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYER 3 OF POST REMEDIATION SAMPLES (POST-R#5)

Samples	FETAX Controls				SAND		KD		ZK		ZL		ZP	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2
Severe														
Stunted														
Gut		1						1	2		8	12	1	3
Edema														
Multiple											3	8		3
Cardiac											1			
Abdominal														
Facial											1			
Cephalic														
Optic														
Tail														
Notochord														
Fin														
Face			1		1		1	1	4	3	11	10	2	3
Eye											2	3		2
Brain														
Hemorrage														
Cardiac														
Blisters														1
Other														
No. Malformed	0	1	1	0	1	0	1	2	4	3	12	13	3	3
No. Scored	30	30	28	29	29	26	13	30	25	27	30	26	28	29

TABLE 73 CONTINUED. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYER 3 OF POST REMEDIATION SAMPLES (POST-R#5)

Samples	ZQ		ZR		ZS		ZU		ZV		ZW		ZX		ZZ		ZZA	
Replicate number	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																		
Stunted				1	2		1											
Gut	2	1	1	1	6	1		1		2	2	2		3	1	1		1
Edema																		
Multiple	1							1		1	1			3				
Cardiac																		
Abdominal																		
Facial	1																	
Cephalic																		
Optic																		
Tail																		
Notochord											1							
Fin											1							
Face	2	2	1	1	3	2	1	1	2	7	2	2	2	4	3	2	1	1
Eye	1									1	1			3				
Brain									1					2				
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	2	3	2	3	6	3	2	1	2	7	3	3	2	4	4	3	1	2
No. Scored	22	15	25	19	26	26	4	13	24	28	28	30	30	30	27	28	11	24

TABLE 74. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2 LAYER 3 OF POST REMEDIATION SAMPLES (POST-R#6)

Samples	FETAX Controls				SAND		KD		ZK		ZP	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2
Severe												
Stunted												
Gut		1			1	1	3	1	1	1	2	3
Edema												
Multiple						1	2			1	2	
Cardiac												
Abdominal												
Facial												
Cephalic												
Optic												
Tail												
Notochord												
Fin												
Face			2		1	3	3	2	4	3	4	4
Eye						1	3		1	1	2	
Brain												
Hemorrage												
Cardiac												
Blisters												
Other												
No. Malformed	0	1	2	0	2	3	4	2	4	3	5	5
No. Scored	29	30	30	29	30	25	25	26	25	27	25	19

TABLE 74. CONTINUED. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2 LAYER 3 OF POST REMEDIATION SAMPLES (POST-R#6)

Samples	ZQ		ZR		ZS		ZT		ZU		ZV		ZW		ZX		ZZ		ZZA	
Replicate number	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																				
Stunted			1																	
Gut	4	3	1	1		1	3	1	1	1	1				1	1		2		1
Edema																				
Multiple	1	3					3	1		1						1				
Cardiac																				
Abdominal																				
Facial																				
Cephalic																				
Optic																				
Tail																				
Notochord																				
Fin											1									
Face	6	10	3	3	2	3	3	1	1	2	1	3	1	3	2	3	1	3	2	2
Eye	1	1	1				2	1		1						1				
Brain																				
Hemorrage																				
Cardiac																				
Blisters																				
Other																				
No. Malformed	9	11	4	3	2	4	3	1	2	2	3	3	1	3	3	3	1	3	2	2
No. Scored	13	29	27	26	25	15	3	1	18	24	21	17	8	17	30	27	16	30	20	23

TABLE 75. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 1 LAYER 3 (POST-R#5)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	8.01	7.81	8.12	7.49	8.41	7.2	8.2	7.4
Sand Controls	8.10	7.90	8.27	7.55	8.49	7.3	8.3	7.3
KD	7.98	7.80	8.12	7.53	8.22	7.3	8.2	7.3
ZK	7.79	7.68	7.90	7.51	8.03	7.3	7.6	7.3
ZL	7.30	7.50	7.79	7.33	7.84	7.2	7.5	7.1
ZM	7.10	7.07	7.34	7.00	7.26	*	*	*
ZO	7.46	7.42	7.92	7.27	8.81	7.1	7.4	*
ZP	7.92	7.59	8.28	7.42	8.27	7.2	7.5	7.3
ZQ	7.92	7.67	8.27	7.38	8.28	7.2	7.7	7.2
ZR	7.87	7.50	8.07	75.2	7.89	6.9	7.4	7.3
ZS	7.88	7.56	8.09	7.47	8.00	7.0	7.6	7.3
ZT	7.81	7.49	8.18	7.41	8.15	7.0	7.5	*
ZU	8.01	7.64	8.42	7.45	8.31	7.0	7.9	7.3
ZV	8.01	7.66	8.29	7.48	8.24	7.1	7.7	7.6
ZW	8.10	7.69	8.43	7.55	8.41	7.2	7.6	7.6
ZX	8.02	7.72	8.35	7.50	8.36	7.2	7.7	7.3
ZZ	7.97	7.71	8.21	7.53	8.20	7.2	7.8	7.3
ZZA	8.07	7.73	8.34	7.56	8.17	7.2	7.9	7.4

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 76. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 2 LAYER 3 (POST-R#6)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	8.09	7.74	8.30	7.48	8.15	6.9	7.7	7.7
Sand Controls	8.17	7.85	8.34	7.51	8.45	7.2	8.1	7.7
KD	7.99	7.77	8.15	7.53	8.26	7.3	8.0	7.6
ZK	7.80	7.69	7.89	7.48	8.00	7.3	7.7	7.5
ZL	7.73	7.55	7.94	7.40	7.86	7.1	7.3	*
ZM	7.14	7.11	7.46	6.97	7.27	*	*	*
ZO	7.48	7.39	7.69	7.21	7.82	*	*	*
ZP	7.93	7.72	8.17	7.36	8.15	7.2	7.7	7.5
ZQ	7.94	7.62	8.29	7.41	8.11	7.1	7.8	7.4
ZR	7.85	7.73	8.14	7.45	7.92	7.1	7.2	7.1
ZS	7.92	7.66	8.10	7.42	7.92	7.1	7.5	7.2
ZT	7.91	7.67	8.22	7.25	7.13	7.1	7.6	7.2
ZU	8.11	7.76	8.42	7.49	8.14	7.2	7.8	7.2
ZV	8.04	7.74	8.33	7.51	8.15	7.2	7.8	7.3
ZW	8.14	7.79	8.44	7.54	8.34	7.2	7.9	7.3
ZX	8.04	7.82	8.40	7.53	8.24	7.2	8.0	7.3
ZZ	7.88	7.77	8.23	7.58	8.07	7.3	7.7	7.4
ZZA	8.09	7.84	8.37	7.66	8.24	7.3	7.8	7.5

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 77. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYER 4 OF POST REMEDIATION SAMPLES (POST-R#7)

Samples	FETAX Controls				SAND		ZK		ZN		ZP	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2
Severe												
Stunted												
Gut						1	1	3	1			
Edema												
Multiple												
Cardiac												
Abdominal												
Facial												
Cephalic												
Optic												
Tail												
Notochord												
Fin												
Face		1			2	2	2	1	3	2	2	1
Eye						1						
Brain												
Hemorrage												
Cardiac												
Blisters												
Other												
No. Malformed	0	1	0	0	2	2	3	3	3	2	2	1
No. Scored	29	30	30	30	30	28	23	29	30	28	30	27

TABLE 77. CONTINUED. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYER 4 OF POST REMEDIATION SAMPLES (POST-R#7)

Samples	ZQ		ZR		ZS		ZU		ZV		ZW		ZX		ZY		ZZ	
Replicate number	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe														1				
Stunted												1						
Gut	2			1					1	1						1		1
Edema																1		
Multiple																		
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic																		
Tail																		
Notochord																		
Fin																		
Face	1	1	1	1	1	2	1	2	3	4	2	1	2	1	1	2	2	1
Eye																1		
Brain																		
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	2	1	1	2	1	2	1	2	3	4	2	2	2	2	1	2	2	2
No. Scored	30	30	30	30	29	27	30	29	29	30	29	25	29	28	27	30	28	30

TABLE 78. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2 LAYER 4 OF POST REMEDIATION SAMPLES (POST-R#8)

Samples	FETAX Controls				SAND		ZK		ZN		ZP	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2
Severe											1	
Stunted												
Gut				2			1	1		3		1
Edema												
Multiple												
Cardiac												
Abdominal												
Facial												
Cephalic												
Optic												
Tail												
Notochord												
Fin												
Face	1	1		3	2	1	1	1	4	5	3	2
Eye						1						
Brain												
Hemorrage												
Cardiac												
Blisters												
Other												
No. Malformed	1	1	0	3	2	2	2	2	4	7	4	3
No. Scored	30	30	29	28	26	28	14	23	20	20	26	24

TABLE 78. CONTINUED. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2 LAYER 4 OF POST REMEDIATION SAMPLES (POST-R#8)

Samples	ZQ		ZR		ZS		ZU		ZV		ZW		ZX		ZY		ZZ	
Replicate number	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																		
Stunted																		
Gut	2		1		1	1	1	2		3	2	4	1	1	3		1	1
Edema																		
Multiple											2	2	1		1			1
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic								1		1				1		2		1
Tail								1		1	1			1		2		1
Notochord																		
Fin																		
Face	3	2	2	2	2	1	2	3	2	4	3	5	3	1	4	1	3	2
Eye	1									1	1	2	1					1
Brain	1		1															
Hemorrage																		
Cardiac																		
Blisters																		
Other												1*						
No. Malformed	3	2	3	2	3	2	2	3	2	4	2	6	3	3	4	3	3	3
No. Scored	24	25	22	11	21	19	21	27	26	25	23	23	21	21	21	25	25	22

* Embryo with 2 heads

TABLE 79. SUMMARY OF RESULTS FROM TEST 1 LAYER 4 OF POST REMEDIATION SOIL SAMPLES (POST-R#7)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	0.83	0.83	0.98
Sand Controls	3.33	6.90	0.98
ZK	13.33	11.54	0.95
ZL	100.0		
ZM	100.0		
ZN	3.33	8.62	0.89
ZP	5.0	5.26	0.95
ZQ	0.0	5.0	0.96
ZR	0.0	5.0	0.95
ZS	6.67	5.36	0.94
ZU	1.67	5.08	0.96
ZV	1.67	11.86	0.94
ZW	10.0	7.41	0.97
ZX	5.0	7.02	0.95
ZY	5.0	5.26	0.94
ZZ	3.33	6.90	0.94

TABLE 80. SUMMARY OF RESULTS FROM TEST 2 LAYER 4 OF POST REMEDIATION SOIL SAMPLES (POST-R#8)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	2.50	4.35	1.03
Sand Controls	10.0	7.42	1.02
ZK	38.33	10.81	0.99
ZL	100.0		
ZM	100.0		
ZN	33.33	27.50	0.93
ZP	16.67	14.00	0.96
ZQ	18.33	10.20	0.99
ZR	45.0	15.15	0.99
ZS	33.33	12.50	1.01
ZU	20.0	10.42	1.02
ZV	15.0	11.76	0.98
ZW	23.33	19.57	0.96
ZX	30.0	14.29	0.99
ZY	23.33	15.22	0.96
ZZ	21.67	12.77	1.02

TABLE 81. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 1 LAYER 4 (PQST-R#7)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	8.3	7.3	8.1	6.9	8.2	7.1	8.0	7.3
Sand Controls	8.2	7.7	8.1	7.2	8.3	7.3	8.2	7.5
ZK	7.8.	7.6	7.7	7.3	7.9	7.3	7.9	7.5
ZL	7.7	7.4	7.4	7.2	7.8	*	*	*
ZM	7.1	7.1	7.1	7.0	7.5	*	*	*
ZN	7.4	7.3	7.2	7.2	7.7	7.3	7.8	7.5
ZP	7.0	7.5	7.4	7.3	8.0	7.4	8.1	7.4
ZQ	7.1	7.6	7.6	7.3	7.2	7.4	8.1	7.4
ZR	7.8	7.1	7.3	7.1	7.4	7.0	7.7	7.0
ZS	8.0	7.4	7.7	7.3	7.8	7.2	7.9	7.2
ZU	8.1	7.5	7.9	7.4	8.0	7.3	8.1	7.3
ZV	8.0	7.6	8.0	7.4	8.1	7.3	8.1	7.4
ZW	8.1	7.7	8.0	7.4	8.2	7.4	8.2	7.4
ZX	8.0	7.7	8.1	7.4	8.2	7.4	8.2	7.5
ZY	7.9	7.7	8.1	7.5	8.2	7.4	8.2	7.5
ZZ	8.0	7.7	8.1	7.5	8.2	7.5	8.2	7.5

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 82. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 2 LAYER 4 (POST-R#8)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	8.2	7.5	8.0	7.1	7.9	7.2	8.1	7.1
Sand Controls	8.3	7.7	8.1	7.2	8.1	7.3	8.2	7.3
ZK	7.8	7.6	8.0	7.3	7.9	7.3	8.0	7.3
ZL	7.4	7.4	7.2	7.2	7.8	*	*	*
ZM	7.2	7.2	7.0	7.0	7.6	*	*	*
ZN	7.3	7.4	7.2	7.1	7.7	7.3	7.9	7.4
ZP	7.7	7.6	7.5	7.2	7.9	7.3	8.1	7.4
ZQ	7.8	7.6	7.4	7.3	8.1	7.3	8.2	7.5
ZR	7.8	7.2	7.5	6.9	7.3	7.3	7.6	7.1
ZS	7.9	7.5	7.5	7.2	7.8	7.3	7.9	7.3
ZU	8.0	7.6	7.7	7.3	8.1	7.4	8.1	7.4
ZV	7.9	7.7	7.8	7.4	8.1	7.4	8.1	7.5
ZW	8.1	7.7	7.5	7.4	8.2	7.4	8.1	7.5
ZX	7.8	7.7	7.8	7.3	8.2	7.4	8.2	7.5
ZY	7.7	7.7	7.6	7.3	8.2	7.4	8.2	7.4
ZZ	8.0	7.8	7.8	7.4	8.3	7.4	8.2	7.5

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 83. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYERS 1 AND 3 OF POST REMEDIATION SAMPLES (POST-R#9)

Samples	FETAX Controls				SAND		ZGA-4		ZK		ZL		ZN		ZY		ZZ	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																		
Stunted															1			
Gut	2	2	1	3	2	4	11	12	16	13	6	2	4	2	2	3	2	3
Edema																		
Multiple	2			2		2				1	1		2				1	
Cardiac																		
Abdominal	1			1														
Facial																		
Cephalic																		
Optic																		
Tail	1			1	1										1			
Notochord	1			1	1										1			
Fin																		
Face	2	2		2	3	2	11	11	14	10	5	2	3	1	2	2	1	1
Eye	2			1		2	1			1	1	1	2				1	
Brain	1																1	
Hemorrage																		
Cardiac																		
Blisters																		
Other																		
No. Malformed	2	2	1	3	6	4	11	12	16	13	7	4	4	2	4	5	2	4
No. Scored	30	29	30	30	30	25	17	16	26	24	22	23	25	14	23	30	11	19

TABLE 84. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2 LAYERS 1 AND 3 OF POST REMEDIATION SAMPLES (POST-R#10)

Samples	FETAX Controls				SAND		ZGA-4		ZK		ZL		ZN		ZR		ZY		ZZ	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																				
Stunted										1										
Gut			1		1	2	12	10	7	4	3	3	3	4	8	5	2	1	1	1
Edema																				
Multiple																				
Cardiac																				
Abdominal																				
Facial																				
Cephalic																				
Optic																				
Tail						1														
Notochord						1														
Fin																				
Face	1				1		13	12	6	2	2	1	6	3	13	10	2	2	2	1
Eye														1						
Brain														1						
Hemorrage																				
Cardiac																				
Blisters																				
Other																				
No. Malformed	1	0	1	0	2	3	13	15	8	6	4	4	8	7	15	10	4	3	3	2
No. Scored	30	30	29	30	23	29	24	30	20	24	30	28	28	29	23	14	28	29	24	26

TABLE 85. SUMMARY OF RESULTS FROM TEST 1 LAYERS 1 AND 3 OF POST REMEDIATION SOIL SAMPLES (POST-R#9)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	0.83	6.72	0.91
Sand Controls	8.33	18.00	0.81
ZGA-1	100.0		
ZGA-2	100.0		
ZGA-3	100.0		
ZGA-4	45.00	69.70	0.79
ZK	16.67	58.00	0.81
ZL	25.00	24.44	0.86
ZM	100.0		
ZP	100.0		
ZQ	100.0		
ZR	100.0		
ZT	0.0	13.33	0.98
ZZ	50.00	20.00	0.90
ZN	35.00	15.38	0.90
ZY	11.67	16.98	0.90

TABLE 86. SUMMARY OF RESULTS FROM TEST 2 LAYERS 1 AND 3 OF POST REMEDIATION SOIL SAMPLES (POST-R#10)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	0.83	1.70	0.93
Sand Controls	13.33	9.52	0.84
ZGA-1	100.0		
ZGA-2	100.0		
ZGA-3	100.0		
ZGA-4	10.00	51.85	0.82
ZK	26.67	31.82	0.84
ZL	3.33	13.79	0.88
ZM	100.0		
ZP	100.0		
ZQ	100.0		
ZR	38.33	67.57	0.81
ZT	15.00	11.76	0.93
ZZ	16.67	10.00	0.89
ZN	5.00	26.32	0.83
ZY	5.00	12.28	0.91

TABLE 87. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 1 LAYERS 1 AND 3 (POST-R#9)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	7.7	7.2	7.9	7.2	7.8	7.1	7.8	7.0
Sand Controls	8.0	7.4	8.1	7.4	8.0	7.2	8.0	7.1
ZGA-1	7.9	7.5	8.0	*	*	*	*	*
ZGA-2	7.8	7.4	7.9	*	*	*	*	*
ZGA-3	7.7	7.4	7.8	7.4	7.9	7.3	8.0	*
ZGA-4	7.8	7.4	7.8	7.4	7.8	7.3	7.9	7.2
ZK	7.8	7.5	7.8	7.4	7.8	7.3	7.9	7.2
ZL	8.1	7.3	7.7	7.4	7.2	7.3	7.6	7.0
ZM	8.0	7.4	7.6	7.3	7.4	7.2	7.7	*
ZN	8.0	7.4	7.8	7.4	7.6	7.2	7.8	7.1
ZP	8.0	7.5	7.8	*	*	*	*	*
ZQ	8.0	7.5	7.9	7.4	7.8	7.3	8.0	7.4
ZR	8.0	7.6	8.0	7.4	7.9	7.3	8.1	7.4
ZY	8.1	7.7	8.1	7.5	8.2	7.4	8.3	7.4
ZZ	8.1	7.7	8.1	7.6	8.2	7.4	8.3	7.3

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 88. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 2 LAYERS 1 AND 3 (POST-R#10)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	7.9	7.2	7.6	7.2	7.7	7.0	7.8	7.1
Sand Controls	8.2	7.5	8.0	7.3	8.0	7.1	8.1	7.1
ZGA-1	8.0	7.5	8.0	*	*	*	*	*
ZGA-2	7.9	7.4	7.8	*	*	*	*	*
ZGA-3	7.8	7.4	7.8	7.3	7.9	7.1	8.0	7.2
ZGA-4	7.8	7.5	7.8	7.4	7.8	7.2	7.9	7.2
ZK	7.8	7.5	7.8	7.4	7.8	7.2	7.9	7.3
ZL	7.7	7.1	7.4	7.2	7.2	7.1	7.1	7.0
ZM	7.7	7.3	7.5	7.2	7.4	7.1	7.3	*
ZN	7.9	7.4	7.7	7.3	7.6	7.2	7.5	7.2
ZP	7.9	7.5	7.8	7.3	7.7	7.2	7.7	*
ZQ	8.0	7.5	7.9	7.4	7.8	7.2	7.8	7.4
ZR	8.0	7.6	8.0	7.4	7.9	7.2	7.9	7.2
ZY	8.1	7.6	8.1	7.5	8.1	7.3	8.1	7.2
ZZ	8.1	7.7	8.1	7.5	8.2	7.3	8.1	7.3

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 89. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 1 LAYERS 2 AND 4 OF POST REMEDIATION SAMPLES (POST-R#11)

Samples	FETAX Controls				SAND		KD		ZO		ZT		ZU		ZV		ZW		ZX		ZZA	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																						
Stunted																						
Gut	2	3	1	1	2	5	2	4	1		7	4	2	1	14	8	5	4	8	1	7	4
Edema																	2	1				4
Multiple	1	3		1							3											
Cardiac												1					1					
Abdominal	1																					
Facial																						
Cephalic																						
Optic																					1	
Tail		1	1																		1	
Notochord			1																			
Fin											6	3	1	1	13	5	5	4	9	2	6	4
Face	2	3	1	1	2	3	1	3	1		3	2					3	2	2		4	4
Eye				1		1		2	1		2	1					1				3	
Brain																						
Hemorrage																						
Cardiac																						
Blisters																						
Other																						
No. Malformed	2	3	3	1	2	5	2	4	1	0	7	4	2	2	15	11	5	4	9	2	7	4
No. Scored	30	30	30	29	25	29	24	26	1	0	24	28	26	23	23	18	24	20	9	2	21	27

TABLE 90. MALFORMATION CHARACTERIZATION AND QUANTIFICATION FOR TEST 2 LAYERS 2 AND 4 OF POST REMEDIATION SAMPLES (POST-R#12)

Samples	FETAX Controls				SAND		KD		ZO		ZT		ZU		ZV		ZW		ZZA	
Replicate number	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Severe																				
Stunted				1					2											
Gut	2			2		2	1	1	4	3	1	2	2	2	1	1		2	1	1
Edema																				
Multiple				1		2				1			1	1				2		
Cardiac																				
Abdominal																				
Facial																				
Cephalic																				
Optic																				
Tail				1																
Notochord				1																
Fin																				
Face		1		1	2	2	2	1	4	3	1	2	2	3	4	5	3	3	2	2
Eye				1		2				1			1	1				2		
Brain																				
Hemorrage																				
Cardiac																				
Blisters																				
Other																				
No. Malformed	2	1	0	2	2	2	2	2	7	3	2	2	2	3	5	5	3	3	2	3
No. Scored	29	30	27	30	19	23	19	22	7	3	29	23	20	22	19	26	21	25	5	18

TABLE 91. SUMMARY OF RESULTS FROM TEST 1 LAYERS 2 AND 4 OF POST REMEDIATION SOIL SAMPLES (POST-R#11)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	0.83	7.53	0.94
Sand Controls	10.00	12.62	0.91
ZS	100.0		
ZU	18.33	8.16	0.88
ZV	31.67	63.41	0.83
ZW	26.67	20.45	0.85
ZX	81.67	100.0	0.77
ZO	98.33	100.0	0.85
ZT	13.33	21.15	0.89
ZZA	20.00	22.92	0.83

TABLE 92. SUMMARY OF RESULTS FROM TEST 2 LAYERS 2 AND 4 OF POST REMEDIATION SOIL SAMPLES (POST-R#12)

Sample	% Mortality	% Malformation	Mean Growth (cm)
FETAX Controls	3.33	4.31	0.94
Sand Controls	30.00	9.52	0.89
ZS	100.0		
ZU	30.0	11.90	0.91
ZV	25.0	22.22	0.89
ZW	23.33	13.04	0.91
ZX	100.0		
ZO	83.33	100.0	0.72
ZT	13.33	7.69	0.92
ZZA	61.67	21.74	0.91

TABLE 93. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 1 LAYERS 2 AND 4 (POST-R#11)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	8.0	7.2	7.7	7.2	7.7	7.2	7.6	7.3
Sand Controls	8.2	7.3	8.0	7.3	7.9	7.2	7.8	7.3
KD	8.2	7.4	8.1	7.3	8.0	7.3	7.9	7.4
ZO	8.2	7.5	8.1	7.4	8.0	7.3	7.9	7.2
ZS	8.2	7.5	8.0	7.4	8.0	7.3	7.9	7.0
ZT	8.3	7.5	8.0	7.3	7.6	7.1	7.3	7.5
ZU	8.3	7.6	8.2	7.4	7.8	7.2	7.5	7.6
ZV	8.2	7.6	8.1	7.4	7.9	7.2	7.6	7.6
ZW	8.3	7.7	8.2	7.4	8.0	7.3	7.7	7.6
ZX	8.3	7.7	8.2	7.4	8.0	7.3	7.8	7.5
ZZA	8.3	7.7	8.2	7.4	8.1	7.3	7.9	7.5

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 94. SUMMARY OF PH VALUES TAKEN DURING THE COURSE OF TEST 2 LAYERS 2 AND 4 (POST-R#12)

Sample	0 hours		24 hours		48 hours		72 hours	
	start	end	start	end	start	end	start	end
FETAX Controls	8.1	7.1	7.8	7.2	7.9	7.1	7.6	7.4
Sand Controls	8.2	7.3	8.0	7.3	8.0	7.2	7.8	7.5
KD	8.2	7.4	7.1	7.2	8.0	7.4	7.9	7.5
ZO	8.1	7.5	7.0	7.4	8.1	*	*	*
ZS	8.1	7.5	7.0	7.3	8.0	*	*	*
ZT	8.0	7.1	7.8	7.4	7.8	7.2	7.8	7.1
ZU	8.1	7.3	8.0	7.5	8.0	7.4	7.9	7.3
ZV	8.1	7.4	8.0	7.5	8.0	7.4	7.9	7.3
ZW	8.1	7.4	8.1	7.5	8.1	7.4	8.0	7.4
ZX	8.1	7.5	8.1	7.5	8.1	7.4	8.0	7.4
ZZA	8.2	7.6	8.1	7.5	8.1	7.5	8.1	7.4

* 100% mortality occurred before 96 hours. Therefore, there was no solution to measure the pH at the end of 96 hours.

TABLE 95. FEMALE REPRODUCTIVE TOXICITY ASSAY: RESULTS FROM POSITIVE CONTROL TEST USING DMDT.

	Control Female		0.001 mg/g DMDT Female	
	pre-exposure	post-exposure	pre-exposure	post-exposure
Male #	45	45	24	24
Egg Weight	4.07	11.26	12.69	2.93
% Normal eggs	60.50	27.00	75.50	5.50
% Fertilized eggs	46.00	34.50	70.00	0.00
% Normally Cleaving eggs	43.50	26.00	33.00	0.00
% Malformed	35.71	*	20.22	*
% Mortality	58.00	*	11.00	*

* Data not collected

TABLE 96. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: FOOD INGESTION DATA OVER JP-4 ORAL EXPOSURE (TOTAL INGESTED GIVEN IN µL OF JP-4)

Week #	Female #6	Female #7	Female #8
1	105 µL	120 µL	150 µL
2	126	140	161
3	22	84	48
4	42	84	22
5	88	105	92
6	140	133	110
7	132	120	110
8	147	133	66

TABLE 97. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: WEIGHT DATA OVER JP-4 ORAL EXPOSURE FOR CONTROL FEMALE FROGS

Week #	Female #1		Female #2		Female #4	
	Weight	% of initial. weight	Weight	% of initial. weight	Weight	% of initial. weight
1	188.8		165.8		165.8	
2	187.0	94.3	154.0	92.9	175.0	105.5
3	187.0	99.0	163.0	98.3	182.2	109.9
4	195.1	103.3	163.1	98.4	182.3	110.0
5	186.4	98.7	165.8	100.0	182.2	109.9
6	176.5	93.5	154.9	93.4	176.3	106.3
7	178.0	94.3	160.3	96.7	177.4	107.0
8	180.3	95.5	166.7	100.5	174.2	105.1
9	182.7	96.8	167.5	101.0	175.9	106.1
10	*	*	*	*	*	*
11	167.1	88.5	175.7	106.0	155.5	93.8

* Data not recorded.

TABLE 98. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: WEIGHT DATA OVER JP-4 ORAL EXPOSURE FOR JP-4 EXPOSED FEMALE FROGS

Week #	Female #6		Female #7		Female #4	
	Weight	% of initial. weight	Weight	% of initial. weight	Weight	% of initial. weight
1	142.0		134.4		165.3	
2	137.0	6.5	131.0	97.5	152.0	92.0
3	146.0	102.8	137.5	102.3	158.2	95.7
4	140.0	98.6	137.9	102.6	149.9	90.7
5	146.9	103.5	137.9	102.6	152.4	92.2
6	135.8	95.6	128.4	95.5	144.9	87.7
7	145.1	102.2	136.1	101.3	146.9	88.9
8	142.9	100.6	129.9	96.7	146.9	88.9
9	150.1	105.7	135.0	100.4	145.4	88.0
10	*	*	*	*	*	*
11	128.0	90.1	127.7	95.0	142.1	86.0
12	127.9	90.1	127.9	95.2	142.1	86.0
13	127.9	90.1	127.9	95.2	135.9	82.2
14	128.6	90.6	128.6	95.7	139.3	84.3
15	131.8	92.8	125.1	93.1	136.6	82.6

* Data not recorded.

TABLE 99. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: WEIGHT DATA OVER JP-4 ORAL EXPOSURE FOR CONTROL MALE FROGS

Week #	Male #12		Male #18		Male #16	
	Weight	% of initial. weight	Weight	% of initial. weight	Weight	% of initial. weight
1	81.8		74.5		64.0	
2	89.0	108.8	78.0	104.7	65.0	101.6
3	92.3	112.8	84.2	113.0	62.4	97.5
4	91.2	111.5	83.6	112.2	67.2	105.0
5	87.9	107.5	78.4	105.2	63.4	99.1
6	82.8	101.2	73.8	99.1	56.0	87.5
7	87.4	106.8	77.7	104.3	64.2	100.3
8	84.1	102.8	78.4	105.2	60.4	94.4
9	86.4	105.6	78.9	105.9	60.9	95.2
10	*	*	*	*	*	*
11	*	*	*	*	*	*
12	84.1	102.8	77.5	104.0	64.3	100.5
13	92.5	113.1	83.9	112.6	59.5	93.0
14	84.0	102.7	76.2	102.3	59.6	93.1
15	79.1	96.7	74.4	99.9	58.8	91.9

* Data not recorded.

TABLE 100. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: WEIGHT DATA OVER JP-4 QRAL EXPOSURE FOR JP-4 EXPOSED MALE FROGS

Week #	Male #15		Male #3		Male #8	
	Weight	% of initial. weight	Weight	% of initial. weight	Weight	% of initial. weight
1	61.7		60.3		64.0	
2	67.0	108.6	65.0	107.8	68.0	106.3
3	63.7	103.2	61.5	102.0	68.8	107.5
4	60.7	98.4	63.5	105.3	67.4	105.3
5	62.3	101.0	58.4	96.8	66.9	104.5
6	57.9	93.8	57.4	95.2	59.1	92.3
7	62.2	100.8	62.9	104.3	59.5	93.0
8	64.6	104.7	56.3	93.4	65.9	103.0
9	65.0	105.3	58.1	96.4	66.8	104.4
10	*	*	*	*	*	*
11	60.3	97.7	*	*	68.3	106.7
12	60.3	97.7	52.6	87.2	68.3	106.7
13	59.6	96.6	57.9	96.0	59.5	93.0
14	77.0	124.8	61.9	102.0	66.2	103.4
15	61.5	99.7	67.5	111.9	65.9	103.0

* Data not recorded.

TABLE 101. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: INITIAL BREEDING AND EGG CLUTCH DATA PRIOR TO JP-4 ORAL EXPOSURE.

	JP-4 Exposed Animals			Control Animals		
Male	15	3	8	12	18	16
Female	6	7	8	1	2	4
Clutch Weight (g)	22.85	22.27	8.20	14.13	17.08	20.00
% Normal	61.50	68.00	72.50	72.00	68.50	85.00
% Fertilized	79.00	72.50	72.00	43.00	65.50	81.05
% Normally Cleaving	55.00	68.50	64.00	42.50	56.00	70.50
% Malformed	23.91	*	40.00	6.57	9.60	*
% Mortality	8.00	0.0	7.5	1.00	1.00	0.0
Mean Embryo Length (cm)	0.926	0.899	0.781	0.922	0.940	0.940

* Data not recorded.

TABLE 102. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: FINAL BREEDING AND EGG CLUTCH DATA AFTER JP-4 ORAL EXPOSURE.

	JP-4 Exposed Animals			Control Animals		
Male	15	3	8	12	18	16
Female	6	7	8	1	2	4
Clutch Weight (g)	14.32	4.17	4.64	20.85	8.06	7.11
% Normal	64.00	76.50	79.00	44.00	78.50	73.50
% Fertilized	72.50	84.50	85.00	39.50	80.50	86.50
% Normally Cleaving	63.50	76.00	79.00	36.00	76.50	70.50
% Malformed	18.08	35.94	26.20	12.90	*	16.23
% Mortality	11.50	4.00	6.50	7.00	*	4.5
Mean Embryo Length (cm)	0.905	0.822	0.879	0.827	0.812	0.928

* Data not recorded.

TABLE 103. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: FINAL BREEDING AND EGG GLUTCH DATA AFTER SCFE EXPOSURE.

Frog No.	Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7
Clutch Weight (g)	*	1.9	3.6	10.2	64 eggs	4.4	19.5
% Normal	*	0.50	1.0	85.0	46.87	2.0	75.00
% Fertilized	*	0.0	0.0	80.0	62.50	0.0	70.00
% Normally Cleaving	*	0.0	0.0	75.0	46.87	0.0	65.00
% Malformed	*	*	*	13.10	14.71	*	8.24
% Mortality	*	*	*	16.0	12.82	*	9.00

Frog No.	S1	S2	S3	S4	S5	S6	S7
Clutch Weight (g)	6.80	3.8	1.00	1.2	1.5	*	5.3
% Normal	78.00	20.00	60.00	45.00	59.00	*	82.00
% Fertilized	84.50	0.00	56.00	0.00	68.00	*	86.50
% Normally Cleaving	74.00	0.00	53.50	0.00	52.00	*	82.00
% Malformed	15.43	*	13.33	*	15.07	*	10.66
% Mortality	12.50	*	87.29	*	63.50	*	1.50

Frog No.	G1	G2	G3	G4	G5	G6	G7
Clutch Weight (g)	*	6.00	*	4.80	2.50	8.00	13.40
% Normal	*	1.00	*	4.50	57.00	53.50	48.00
% Fertilized	*	0.00	*	2.50	64.50	77.00	70.50
% Normally Cleaving	*	0.00	*	2.50	55.55	53.50	48.00
% Malformed	*	*	*	61.90	20.32	12.23	66.67
% Mortality	*	*	*	58.00	6.50	6.00	76.00

Frog No.	O1	O2	O3	O4	O5	O6	O7
Clutch Weight (g)	2.70	4.80	**	12.00	9.80	*	12 eggs
% Normal	0.00	0.00	**	12.00	43.00	*	50.00
% Fertilized	0.00	2.50	**	12.50	60.50	*	0.00
% Normally Cleaving	0.00	0.00	**	10.00	47.00	*	0.00
% Malformed	*	*	**	6.43	20.00	*	*
% Mortality	*	*	**	30.00	50.00	*	*

Frog No.	B1	B2	B3	B4	B5	B6	B7
Clutch Weight (g)	6.30	14.30	0.50	9.00	5.90	9.00	8.00
% Normal	18.00	33.50	45.60	63.00	49.00	52.00	25.00
% Fertilized	13.50	41.50	48.80	81.50	31.50	65.71	35.50
% Normally Cleaving	11.50	31.50	45.60	61.50	25.00	52.00	25.00
% Malformed	24.00	14.11	14.29	20.00	56.25	30.89	21.21
% Mortality	20.00	18.50	30.00	50.00	84.00	38.50	67.00

* Not enough eggs to calculate this endpont
** Frog removed from exposure because it wouldn't eat

TABLE 104. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: ORGAN TO BODY WEIGHT RATIO AFTER SCFE EXPOSURE.

Frog No.	Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7
Body Weight	117.00	127.50	106.80	102.40	105.2	94.6	90.00
lung	0.00684	0.00627	0.00655	0.00684	0.00760	0.00634	0.00556
liver	0.04701	0.05412	0.04401	0.04492	0.05038	0.04017	0.03667
ovary	0.11538	0.12706	0.07959	0.13672	0.06369	0.08774	0.16222
spleen	0.00096	0.00035	0.00040	0.00060	0.00039	0.00053	0.00051

Frog No.	S1	S2	S3	S4	S5	S6	S7
Body Weight	132.1	118.2	109.00	94.6	97.10	87.7	94.80
lung	0.00681	0.00761	0.00826	0.00634	0.00618	0.00684	0.00527
liver	0.04693	0.06007	0.04771	0.02326	0.02472	0.03649	0.03059
ovary	0.09841	0.12014	0.09174	0.10148	0.11125	0.11403	0.09916
spleen	0.00047	0.00054	0.00048	0.00108	0.00033	0.00052	0.00034

Frog No.	G1	G2	G3	G4	G5	G6	G7
Body Weight	133.1	127.3	133.8	108.4	118.2	94.3	94.4
lung	0.00676	0.00707	0.01142	0.00738	0.00592	0.00742	0.00847
liver	0.03606	0.04635	0.06854	0.03506	0.05330	0.04454	0.04449
ovary	0.18407	0.13826	0.06151	0.21771	0.22420	0.12725	0.18432
spleen	0.00050	0.00143	0.00202	0.00073	0.00061	0.00125	0.00068

Frog No.	O1	O2	O3	O4	O5	O6	O7
Body Weight	119.2	91.3	**	89.4	75.5	80.2	85.1
lung	0.00755	0.00876	**	0.00895	0.00795	0.00748	0.00705
liver	0.03691	0.03067	**	0.03356	0.04636	0.05112	0.05170
ovary	0.09648	0.11939	**	0.12192	0.12980	0.07107	0.11868
spleen	0.00051	0.00092	**	0.00132	0.00085	0.00168	0.00188

Frog No.	B1	B2	B3	B4	B5	B6	B7
Body Weight	124.1	119.4	96.4	104.8	82.3	95.5	94.5
lung	0.00645	0.00754	0.01245	0.00668	0.00851	0.00628	0.00847
liver	0.04593	0.04271	0.07469	0.04580	0.05711	0.03770	0.05926
ovary	0.13618	0.14657	0.04046	0.15363	0.13366	0.18848	0.70265
spleen	0.00042	0.00046	0.00148	0.00060	0.00036	0.00057	0.00082

** Frog removed from exposure due to failure to eat

TABLE 105. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: OVARY DATA AFTER SCFE EXPOSURE.

Frog No.	Con 1	Con 2	Con 3	Con 4	Con 5	Con 6	Con 7
% Stage 1	4.61	29.57	49.44	50.4	25.00	50.72	32.56
% Stage 2	16.06	19.13	24.16	21.24	20.00	15.22	23.26
% Stage 3	13.87	20.00	10.67	7.96	5.00	7.25	20.93
% Stage 4	10.95	13.04	10.11	11.50	20.00	12.32	13.18
% Stage 5	10.21	7.83	4.49	11.50	10.00	7.25	8.53
% Stage 6	8.03	6.09	4.49	7.08	10.00	5.80	8.53
% Necrotic	6.57	7.35	2.25	4.42	10.00	1.45	0.78
% Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total # Counted	137	115	178	113	*	138	129

Frog No.	S1	S2	S3	S4	S5	S6	S7
% Stage 1	50.30	50.00	39.44	43.00	46.64	23.44	47.06
% Stage 2	14.75	19.84	7.04	26.57	11.16	28.12	15.69
% Stage 3	10.06	9.52	9.86	9.18	8.76	17.19	10.59
% Stage 4	8.88	3.97	25.35	2.90	11.95	10.94	7.06
% Stage 5	10.06	6.35	6.57	33.82	20.72	6.25	3.92
% Stage 6	0.00	2.38	7.51	0.00	0.00	11.72	15.69
% Necrotic	2.96	7.94	4.23	3.86	0.80	2.34	0.00
% Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total # Counted	169	126	213	2.07	251	256	255

Frog No.	G1	G2	G3	G4	G5	G6	G7
% Stage 1	31.56	45.00	80.00	50.40	60.64	58.25	53.65
% Stage 2	14.34	29.12	5.00	16.98	20.21	11.65	27.37
% Stage 3	31.15	7.06	5.00	13.00	7.80	11.17	3.65
% Stage 4	9.02	5.00	4.00	4.77	1.06	6.80	4.74
% Stage 5	6.15	4.12	1.00	1.33	2.13	3.40	3.65
% Stage 6	2.05	2.06	0.00	0.00	2.48	1.94	1.82
% Necrotic	5.74	2.35	5.00	12.47	5.67	2.91	2.92
% Other	0.00	5.29	0.00	1.06	0.00	3.88	2.19
Total # Counted	244	340	*	377	282	206	274

Frog No.	O1	O2	O3	O4	O5	O6	O7
% Stage 1	49.59	48.11	**	45.22	53.52	60.47	55.93
% Stage 2	14.88	16.49	**	11.03	15.96	9.69	9.75
% Stage 3	9.50	13.75	**	20.22	11.74	7.36	6.36
% Stage 4	13.22	5.84	**	9.56	10.33	11.24	17.80
% Stage 5	9.50	6.87	**	6.25	8.45	2.71	4.66
% Stage 6	2.48	4.81	**	6.62	0.00	0.00	0.85
% Necrotic	0.83	4.12	**	1.10	0.00	8.53	4.66
% Other	0.00	0.00	**	0.00	0.00	0.00	0.00
Total # Counted	242	291	**	272	213	258	236

Frog No.	B1	B2	B3	B4	B5	B6	B7
% Stage 1	56.88	58.65	70.00	52.57	55.82	38.99	53.22
% Stage 2	15.22	11.39	10.00	17.79	6.43	21.30	11.59
% Stage 3	9.78	7.59	4.00	7.90	8.83	9.39	10.73
% Stage 4	5.07	9.28	4.00	3.56	8.83	5.78	9.87
% Stage 5	7.61	7.59	2.00	5.93	10.84	10.47	6.87
% Stage 6	0.00	4.22	0.00	0.79	4.42	11.55	0.00
% Necrotic	5.43	1.27	10.00	11.46	4.82	2.53	7.73
% Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total # Counted	276	237	*	253	249	277	233

* Percentages estimated due to ovary structure ** Frog removed from exposure due to failure to eat

TABLE 106. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: INITIAL BREEDING AND EGG CLUTCH DATA PRIOR DIRECT EXPOSURE TO EGLIN AFB SOIL

Frog No.	K1	K2	K3	B1	B2	B3
Male	18	44	23	26	56	34
Clutch Weight (g)	2.64	14.07	5.07	5.72	14.50	8.94
% Normal	46.00	54.50	75.00	66.50	59.50	70.50
% Fertilized	31.50	46.50	77.00	72.50	39.00	57.00
% Normally Cleaving	26.50	40.50	69.50	54.00	37.00	41.50
% Malformed	33.33	52.17	28.81	38.30	16.39	21.05
% Mortality	35.50	65.50	41.00	29.50	8.50	45.71

Frog No.	O1	O2	O3	G1	G2	G3
Male	12	31	*	9	53	25
Clutch Weight (g)	12.05	13.41	*	15.00	5.86	5.41
% Normal	73.50	44.0	*	77.50	86.00	66.50
% Fertilized	84.00	36.00	*	79.00	84350	72.00
% Normally Cleaving	71.50	31.50	*	72.50	79.00	60.00
% Malformed	10.81	13.29	*	44.90	8.06	32.58
% Mortality	26.00	13.50	*	75.50	7.00	11.00

Frog No.	S1	S2	S3	CON1	CON2	CON3
Male	20	54	57	*	13	58
Clutch Weight (g)	7.93	12.61	5.8	*	2.89	10.03
% Normal	53.50	75.50	75.00	*	66.50	95.50
% Fertilized	48.50	72.00	57.50	*	15.50	98.50
% Normally Cleaving	32.50	62.50	54.00	*	64.00	95.50
% Malformed	36.76	7.82	17.39	*	18.00	10.10
% Mortality	66.00	10.50	31.00	*	50.00	41.00

* Data not recorded

TABLE 107. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: FINAL BREEDING AND EGG CLUTCH DATA AFTER DIRECT EXPOSURE TO EGLIN AFB SOIL

Frog No.	K1	K2	K3	B1	B2	B3***
Male	18	44	23	26	56	*
Clutch Weight (g)	3.55	1.3	1.4	**	3.17	*
% Normal	0.50	71.50	23.50	**	14.00	*
% Fertilized	0.00	76.50	3.50	**	10.00	*
% Normally Cleaving	0.00	69.50	3.50	**	10.00	*
% Malformed	**	34.12	0.0	**	60.00	*
% Mortality	**	15.00	23.53	**	50.00	*

Frog No.	O1	O2	O3***	G1	G2	G3
Male	12	31	*	9	53	25
Clutch Weight (g)	9.18	**	*	8.12	8.47	0.47
% Normal	19.50	**	*	1.00	2.00	38.99
% Fertilized	0.50	**	*	0.00	0.00	29.50
% Normally Cleaving	0.50	**	*	0.00	0.00	25.00
% Malformed	17.24	**	*	**	**	100.00
% Mortality	17.14	**	*	**	**	60.00

Frog No.	S1	S2	S3	CON1	CON2	CON3
Male	20	54	57	*	13	58
Clutch Weight (g)	**	2.60	2.92	*	6.21	5.43
% Normal	**	6.5	28.00	*	52.50	60.50
% Fertilized	**	0.00	42.00	*	63.00	64.00
% Normally Cleaving	**	0.00	26.00	*	51.50	60.50
% Malformed	**	**	25.97	*	14.36	10.38
% Mortality	**	**	23.00	*	9.50	8.5

* Data not recorded
** Not enough eggs to count this endpoint
*** Frog died during exposure

TABLE 108. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: RATIO OF ORGAN WEIGHT TO BODY WEIGHT AFTER EXPOSURE TO EGLIN AFB SOIL.

Female	Body Weight	Lung	Liver	Ovary	Spleen
CON1					
CON2	84.77	0.00755	0.03645	0.11572	0.00032
CON3	79.76	0.00589	0.05529	0.14531	0.00046
K1	89.21	0.00830	0.03878	0.06143	0.00058
K2	55.83	0.00900	0.04729	0.062538	0.00056
K3	105.75	0.00823	0.02998	0.09456	0.00076
B1	113.66	0.00871	0.05666	0.02639	0.00055
B2	64.95	0.00831	0.07883	0.05081	0.00068
B3***					
O1	112.22	0.00561	0.05453	0.11745	0.000356
O2	130.60	0.00904	0.04556	0.06485	0.00051
O3***					
G1	82.23	0.00596	0.06433	0.07868	0.00103
G2	64.00	0.00844	0.06172	0.09781	0.00042
G3	65.86	0.00805	0.04859	0.05314	0.00037
S1	101.27	0.00553	0.05322	0.06280	0.00119
S2	105.83	0.00850	0.03978	0.10111	0.00047
S3	103.30	0.00571	0.05111	0.13930	0.00033

* Data not recorded
*** Frog died during exposure

TABLE 109. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: PERCENT OF OOCYTES IN DUMONT STAGES 1-6 IN OVARIES AFTER EXPOSURE TO EGLIN AFB SOIL

Females	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Necrotic	Other	total counted	% pre-vitellogenic	% vitellogenic
CON1											
CON2	21.28	8.51	15.96	0.65	25.53	18.09	1.06		94	29.79	68.23
CON3	11.22	15.31	15.31	16.33	12.24	17.35	12.24		98	26.53	61.22
K1	21.43	18.37	11.22	15.31	10.20	12.24	2.04		98	39.80	48.98
K2	17.00	31.00	11.00	17.00	10.00	12.00	2.00		100	48.00	50.00
K3	14.00	7.00	6.00	14.00	37.00	11.00	9.00		100	23.00	68.00
B1	69.70	14.14	7.07	2.02	2.02	0.00	5.05		99	83.84	11.11
B2	33.66	25.74	9.90	16.83	8.91	9.90	3.96		101	59.41	45.54
B3***	*	*	*	*	*	*	*	*	*	*	*
O1	19.39	16.33	15.31	12.24	13.27	13.27	10.20	0	98	35.71	54.08
O2	36.08	28.87	10.31	9.28	9.28	5.15	10.3	0	97	64.95	34.02
O3***	*	*	*	*	*	*	*	*	*	*	*
G1	34.31	25.49	13.73	9.80	14.71	1.96	0	0	102	59.80	40.20
G2	14.14	26.26	8.08	8.08	22.22	2.02	19.19	0	99	40.40	40.40
G3	52.00	21.00	5.00	2.00	11.00	0	9.00	0	100	73.00	18.00
S1	54.55	17.17	3.03	6.06	6.06	3.03	10.10	0	99	71.72	18.18
S2	39.81	17.48	10.68	10.68	6.80	4.85	9.71	0	103	57.28	33.01
S3	34.07	6.59	16.48	16.48	10.99	5.49	9.89	0	91	40.66	49.45

* Data not recorded
*** Frog died during exposure

TABLE 110. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: FINAL BREEDING AND EGG CLUTCH DATA AFTER SECOND DIRECT EXPOSURE.

Control Animals	4	13	17	21	22	24	26
Clutch Weight (g)	6.0	3.8	11.0	14.2	7.5	1.9	8.8
% Normal	86.00	59.00	51.00	32.00	11.00	19.50	67.50
% Fertilized	92.50	64.00	53.50	37.50	11.50	11.00	65.50
% Normally Cleaving	84.50	58.00	47.50	30.50	8.50	10.50	58.50
% Malformed	2.60	4.70	8.80	3.10	1.90	0.00	13.30
% Mortality	3.00	4.00	14.50	4.50	8.82	9.38	17.50

ZGA Site Animals	1	5	8	14	15	27	37
Clutch Weight (g)	3.1	3.3	3.9	7.2	*	6.8	8.0
% Normal	32.50	85.50	68.00	14.50	*	25.00	26.50
% Fertilized	36.00	86.50	81.00	6.00	*	18.500	34.00
% Normally Cleaving	26.00	82.50	66.00	6.00	*	16.50	24.00
% Malformed	8.40	7.06	13.60	9.80	*	**	**
% Mortality	28.50	8.00	15.00	2.90	*	16.50	9.50

ZX Site Animals	6	16	19	28	30	34	42
Clutch Weight (g)	7.4	***	3.7	8.0	4.8	5.9	15.3
% Normal	5.050	***	34.50	4.50	84.50	83.50	30.00
% Fertilized	36.50	***	36.50	0.00	86.50	87.50	28.50
% Normally Cleaving	33.00	***	31.00	0.00	79.00	83.50	25.50
% Malformed	6.86	***	56.44	100.00	4.12	2.70	2.60
% Mortality	12.50	***	46.84	66.67	15.00	7.50	4.00

ZP Site Animals	7	10	32	41	44	52	54
Clutch Weight (g)	18.20	11.2	*	6.5	3.3	3.9	0.4
% Normal	9.50	86.00	*	64.50	0.50	65.00	0.00
% Fertilized	4.50	94.00	*	58.50	*	69.00	3.50
% Normally Cleaving	4.00	76.00	*	47.50	*	59.50	0.00
% Malformed	2.22	2.04	*	4.66	*	3.83	*
% Mortality	59.90	2.00	*	3.50	*	8.50	*

KD Site Animals	40	45	57	65	61	63	69
Clutch Weight (g)	5.9	4.6	10.9	5.0	11.6	5.8	0.9
% Normal	84.00	80.50	31.50	88.00	56.50	89.50	31.00
% Fertilized	90.00	77.50	24.50	88.00	48.50	93.50	26.00
% Normally Cleaving	56.50	58.50	19.50	74.00	36.50	75.50	20.00
% Malformed	46.81	2.54	3.61	3.61	22.70	10.99	12.12
% Mortality	53.00	1.50	3.00	3.00	18.50	9.00	29.03

ZO Site Animals	58	62	64	59	66	68	70
Clutch Weight (g)	13.0	5.4	3.2	13.3	4.8	13.8	3.2
% Normal	13.00	28.00	92.00	73.00	17.00	66.50	72.00
% Fertilized	12.00	21.50	99.50	74.50	8.50	52.50	71.00
% Normally Cleaving	8.50	17.50	91.50	64.50	5.50	4.50	56.50
% Malformed	9.30	10.36	17.99	5.64	9.71	28.71	7.25
% Mortality	14.00	3.50	5.50	2.50	5.50	49.50	3.50

* Not enough eggs to calculate this endpont
** This data point not collected
*** Frog died during exposure

TABLE 111. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: ORGAN TO BODY WEIGHT RATIO AFTER SECOND DIRECT EXPOSURE.

Control Animals	4	67	17	21	22	24	36
Body Weight	62.1	58.6	70.3	56.7	60.1	62.2	72.2
lung	0.00088	0.01371	0.00618	0.00087	0.01050	0.00788	0.00862
liver	0.07407	0.04266	0.04552	0.04762	0.05491	0.05145	0.06371
ovary	0.04509	0.05973	0.06857	0.07231	0.09983	0.07074	0.09557
spleen	0.00105	0.00068	0.00082	0.00081	0.00067	0.00086	0.00036

ZGA Site Animals	1	5	8	14	15	27	37
Body Weight	70.3	72.7	64.0	61.4	56.3	65.2	87.4
lung	0.0060	0.00700	0.00540	0.00520	0.00640	0.00640	0.00520
liver	0.86770	0.06050	0.05310	0.05700	0.05680	0.06900	0.05610
ovary	0.07680	0.12380	0.13910	0.06840	0.12430	0.09660	0.04460
spleen	0.00520	0.00540	0.00090	0.00100	0.00080	0.00110	0.00050

ZX Site Animals	6	16	19	28	30	34	42
Body Weight	70.0	*	54.1	67.7	78.6	55.1	82.3
lung	0.01229	*	0.01014	0.00990	0.00625	0.0078	0.00895
liver	0.06143	*	0.04621	0.05022	0.06361	0.05082	0.06440
ovary	0.07714	*	0.09612	0.07238	0.03181	0.05445	0.05225
spleen	0.00109	*	0.00057	0.00050	0.00065	0.00056	0.00098

ZP Site Animals	7	10	32	41	44	52	54
Body Weight	53.6	54.7	89.0	67.2	81.9	70.0	53.3
lung	0.01219	0.01340	0.00811	0.00839	0.01077	0.00688	0.01283
liver	0.06343	0.04205	0.04382	0.04167	0.04029	0.08000	0.04315
ovary	0.07463	0.05302	0.05281	0.08482	0.06716	0.05571	0.07692
spleen	0.00075	0.00067	0.00084	0.00111	0.00106	0.00090	0.00118

KD Site Animals	40	45	57	65	61	63	69
Body Weight	75.0	82.7	61.2	60.2	67.8	79.5	60.4
lung	0.00863	0.01138	0.00915	0.01433	0.00826	0.00826	0.00666
liver	0.06133	0.05804	0.05719	0.06645	0.05162	0.04654	0.05298
ovary	0.09333	0.03023	0.08333	0.03987	0.07522	0.07170	0.06291
spleen	0.00079	0.00097	0.00073	0.00087	0.00066	0.00057	0.00076

ZO Site Animals	58	62	64	59	66	68	70
Body Weight	66.9	56.4	59.8	62.6	62.5	60.5	67.0
lung	0.00900	0.00999	0.00753	0.01046	0.01235	0.00863	0.00933
liver	0.05082	0.04433	0.0551	0.05911	0.03360	0.05785	0.04776
ovary	0.06428	0.09752	0.09031	0.09105	0.09440	0.10248	0.07463
spleen	0.00090	0.00066	0.00403	0.00052	0.00050	0.00058	0.00063

* Frog died during exposure

TABLE 112. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: OVARY DATA AFTER SECOND DIRECT EXPOSURE.

Control Animals	4	13	67	21	22	24	36
% Stage 1	52.71	61.72	69.76	62.07	54.10	63.75	57.73
% Stage 2	22.02	18.66	14.94	13.03	17.21	16.31	19.24
% Stage 3	15.88	4.78	4.84	11.11	8.61	4.23	5.99
% Stage 4	5.78	8.13	2.82	8.05	10.25	6.65	5.68
% Stage 5	2.89	4.31	3.69	5.75	9.02	5.44	7.26
% Stage 6	0.72	1.44	0.00	0.00	0.82	3.02	3.79
% Necrotic	0.00	0.96	4.03	0.00	0.00	0.60	0.32
% Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total # Counted	277	209	248	261	244	331	317

ZGA Site Animals	1	5	8	14	15	27	37
% Stage 1	43.90	53.85	59.66	63.33	32.54	51.85	64.42
% Stage 2	19.51	14.90	17.23	21.25	19.53	17.99	13.11
% Stage 3	9.15	9.62	10.08	5.83	11.24	10.58	8.99
% Stage 4	17.68	7.69	7.56	4.17	12.43	12.70	6.74
% Stage 5	3.05	8.17	4.20	5.00	21.30	5.29	6.37
% Stage 6	6.71	5.77	1.26	0.42	2.37	1.06	0.37
% Necrotic	0.00	0.00	0.00	0.00	0.59	0.53	0.00
% Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total # Counted	164	208	238	240	169	189	267

ZX Site Animals	6	16	19	28	30	34	42
% Stage 1	62.55	*	45.25	35.80	53.05	46.25	55.82
% Stage 2	16.73	*	19.55	26.54	29.11	23.13	20.88
% Stage 3	8.73	*	16.20	16.05	6.10	13.36	9.24
% Stage 4	4.36	*	5.59	15.43	7.98	9.77	7.63
% Stage 5	6.18	*	11.17	4.94	3.29	6.19	6.02
% Stage 6	0.73	*	2.23	1.23	0.47	1.30	0.40
% Necrotic	0.73	*	0.00	0.00	0.00	0.00	0.00
% Other	0.00	*	0.00	0.00	0.00	0.00	0.00
Total # Counted	275	*	179	162	213	307	249

ZP Site Animals	7	10	32	41	44	52	54
% Stage 1	49.21	44.53	44.75	28.00	37.18	40.80	35.94
% Stage 2	32.46	34.01	28.31	24.00	22.38	25.71	21.88
% Stage 3	10.47	15.79	16.44	16.00	16.97	16.27	20.31
% Stage 4	4.71	5.26	7.76	19.33	14.08	12.26	6.77
% Stage 5	2.62	0.40	2.28	8.00	6.14	2.83	4.17
% Stage 6	0.52	0.00	0.46	4.67	3.25	2.12	0.52
% Necrotic	0.00	0.00	0.00	0.00	0.00	0.00	10.42
% Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total # Counted	191	247	219	150	277	424	192

KD Site Animals	40	45	57	65	61	63	69
% Stage 1	40.26	46.25	40.20	46.84	63.76	51.35	53.85
% Stage 2	26.62	32.50	27.45	26.58	23.58	27.48	18.68
% Stage 3	16.23	11.88	23.04	6.33	6.55	9.01	7.69
% Stage 4	12.34	8.75	6.37	10.76	4.37	8.11	12.64
% Stage 5	3.90	0.63	2.45	7.59	0.44	4.05	4.40
% Stage 6	0.65	0.00	0.49	1.90	0.87	0.00	2.20
% Necrotic	0.00	0.00	0.00	0.00	0.44	0.00	0.55
% Other	0.00	0.00	0.00	0.00	0.00	0.00	00.00
Total # Counted	154	160	204	158	229	222	182

TABLE 112. CONTINUED. FEMALE REPRODUCTIVE TOXICITY EXPERIMENT: OVARY DATA AFTER SECOND DIRECT EXPOSURE.

ZO Site Animals	58	62	64	59	66	68	70
% Stage 1	53.70	50.24	42.27	58.37	54.94	41.88	57.75
% Stage 2	19.07	19.32	28.18	15.38	22.22	23.93	18.31
% Stage 3	8.17	9.18	21.36	9.50	6.17	14.81	11.27
% Stage 4	10.12	9.66	5.45	4.52	7.41	13.96	7.04
% Stage 5	7.78	9.66	2.27	7.69	6.17	3.70	4.23
% Stage 6	1.17	1.93	0.45	4.52	2.47	1.71	1.41
% Necrotic	0.00	0.00	0.00	0.00	0.62	0.00	0.00
% Other	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total # Counted	257	207	220	221	162	351	213

* Frog died during exposure

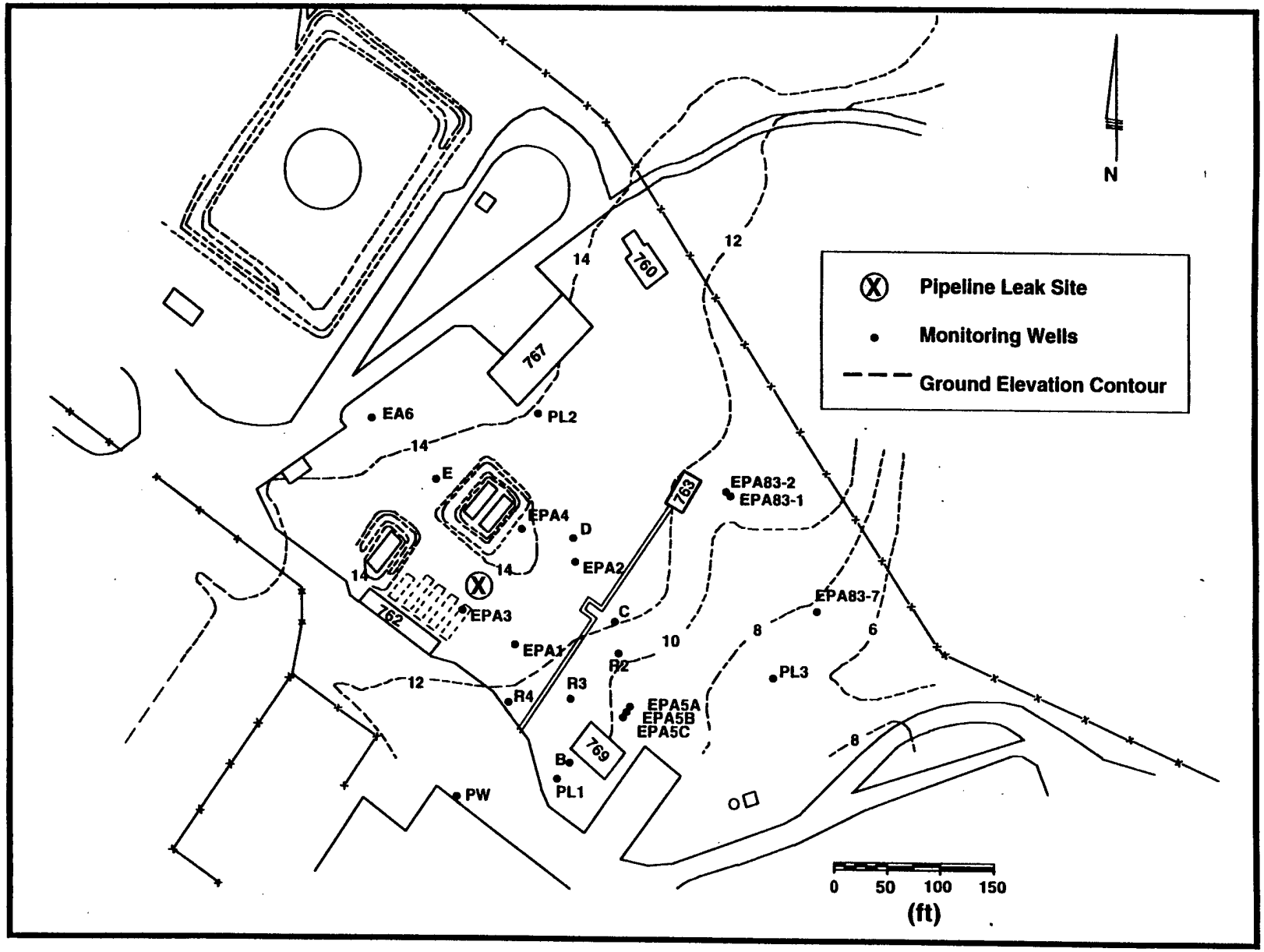


FIGURE 1. SITE MAP OF THE JP-4 SPILL SITE AT EGLIN AIR FORCE BASE.

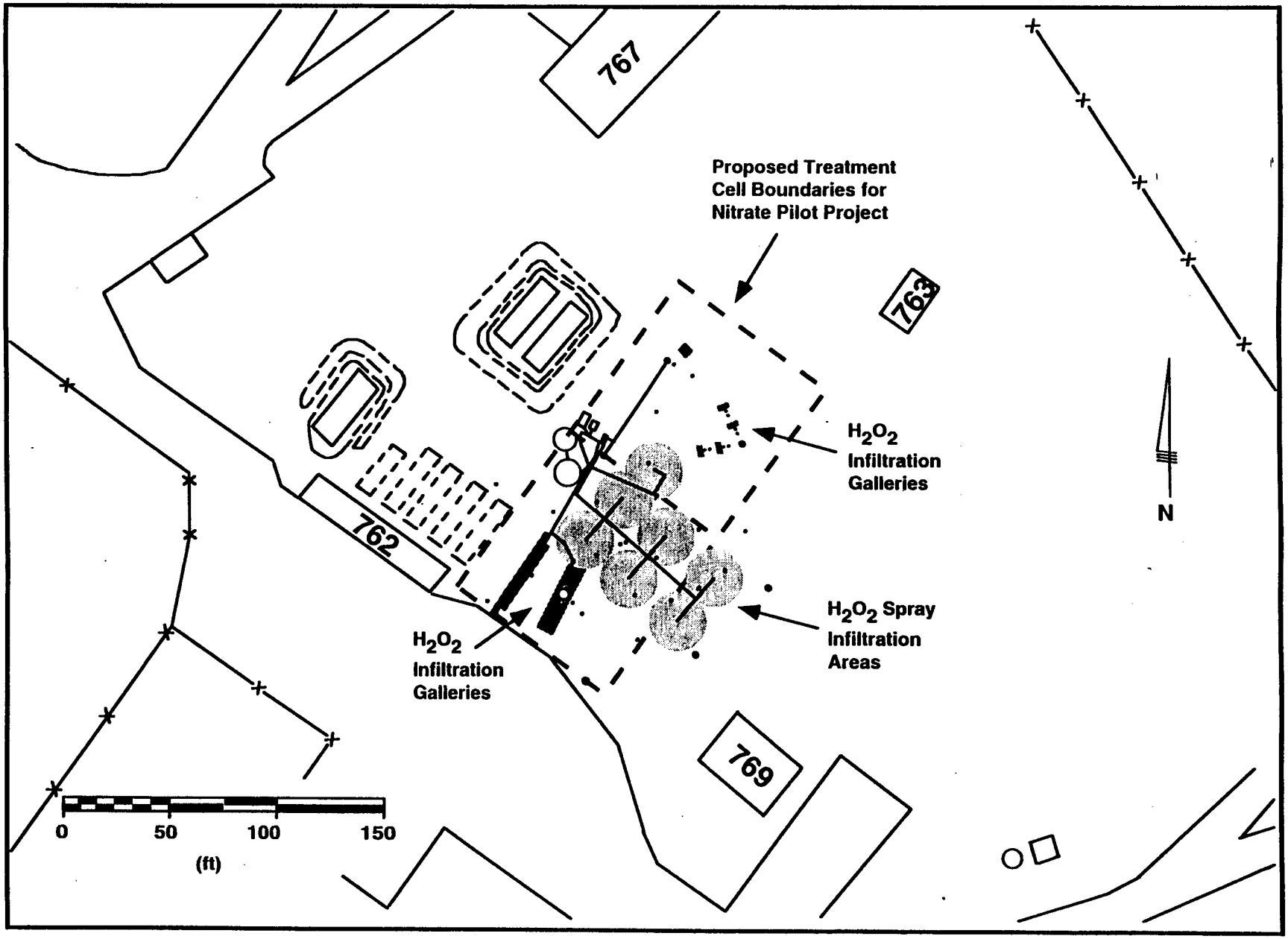


FIGURE 2. MAP OF THE H_2O_2 APPLICATION PLAN OF THE 1986 REMEDIATION ATTEMPT.

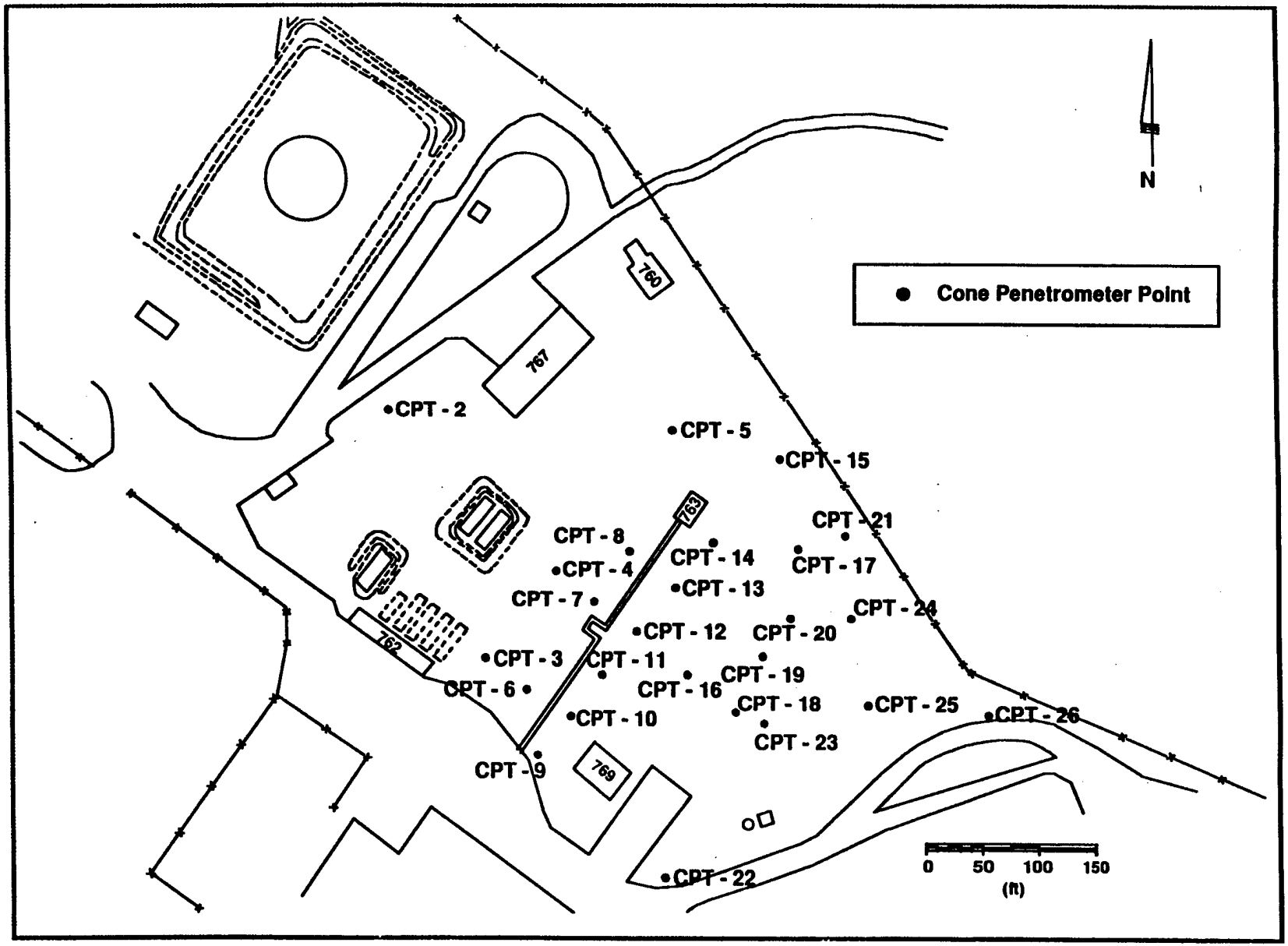


FIGURE 3. LOCATION OF CONE PENETROMETER SAMPLING POINTS TO MEASURE BTEX AND DISSOLVE OXYGEN CONCENTRATIONS.

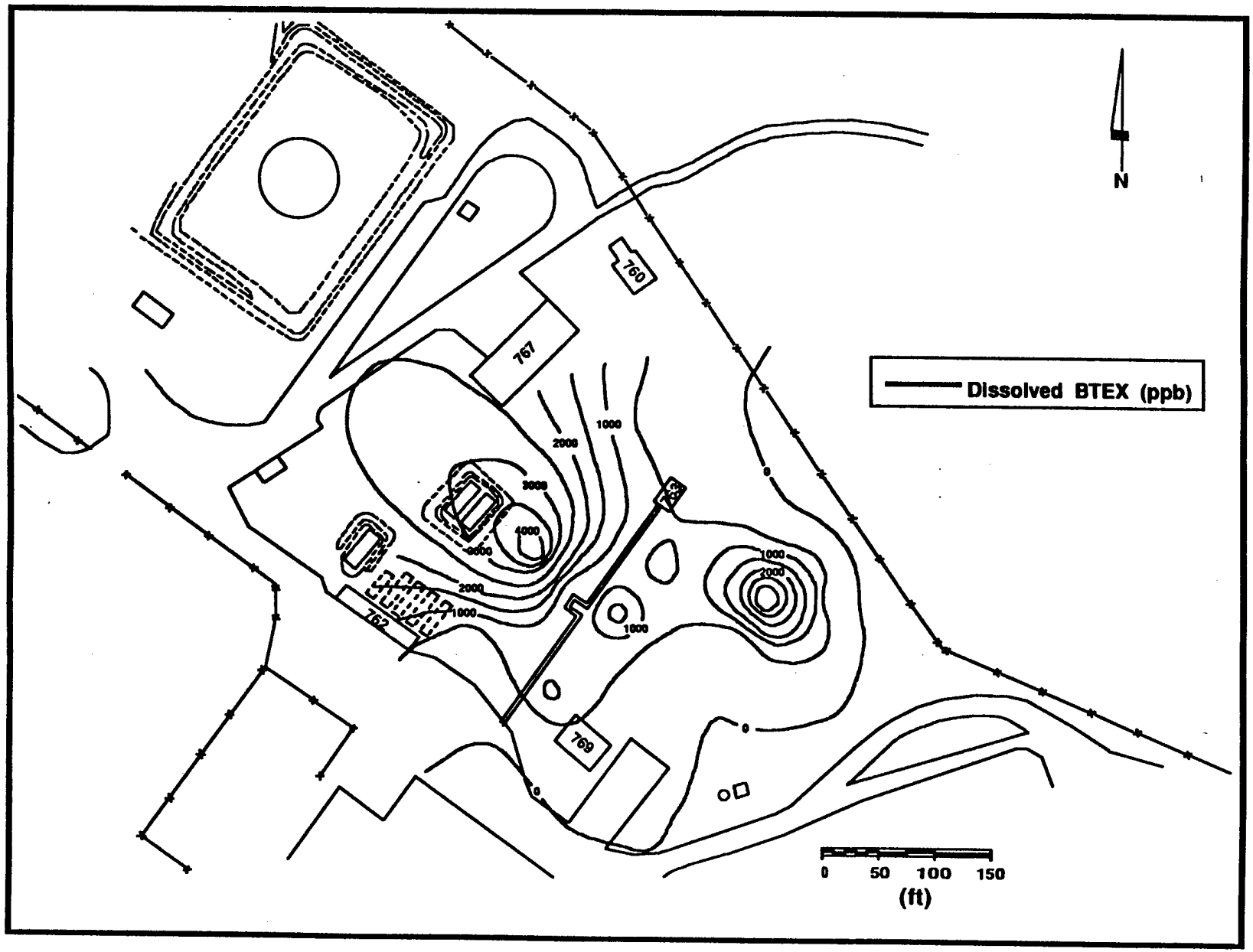


FIGURE 4. CONTOUR MAP OF BTEX CONCENTRATION AT SITE.

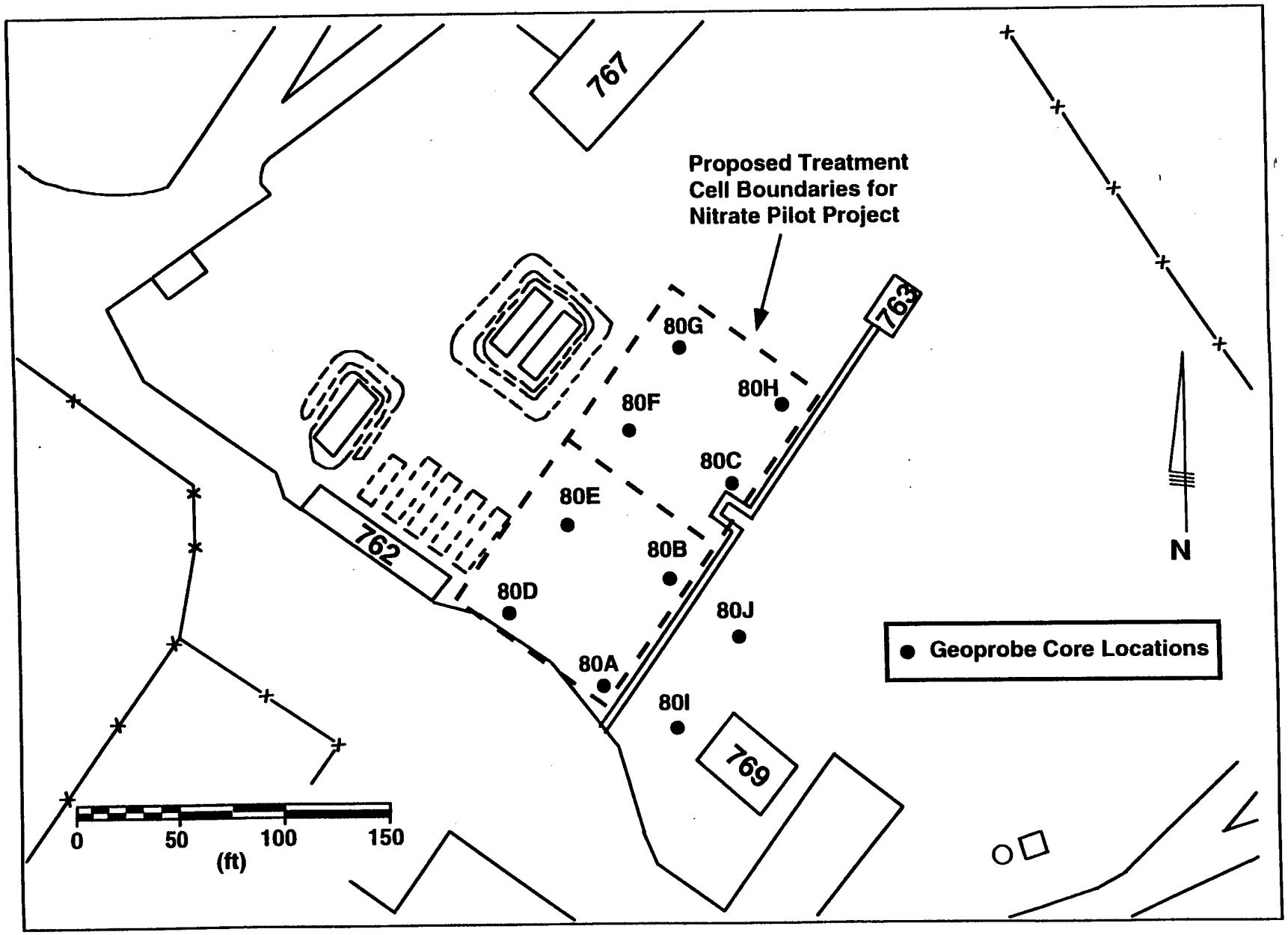


FIGURE 5. LOCATION OF GEOPROBE SAMPLE POINTS FOR WATER QUALITY.

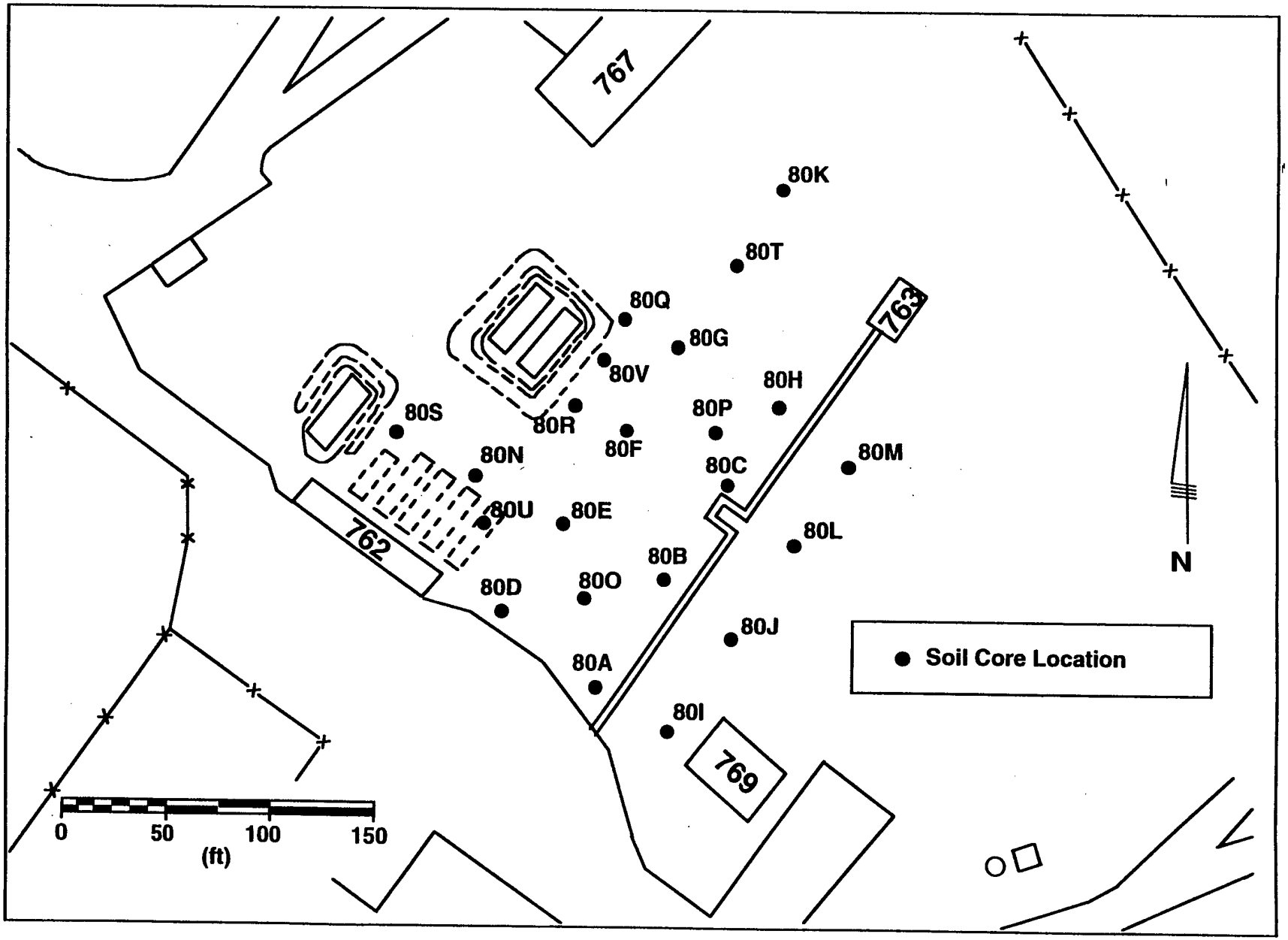


FIGURE 6. LOCATION OF CORE SAMPLES USED IN SITE ANALYSIS PRIOR TO START OF REMEDIATION.

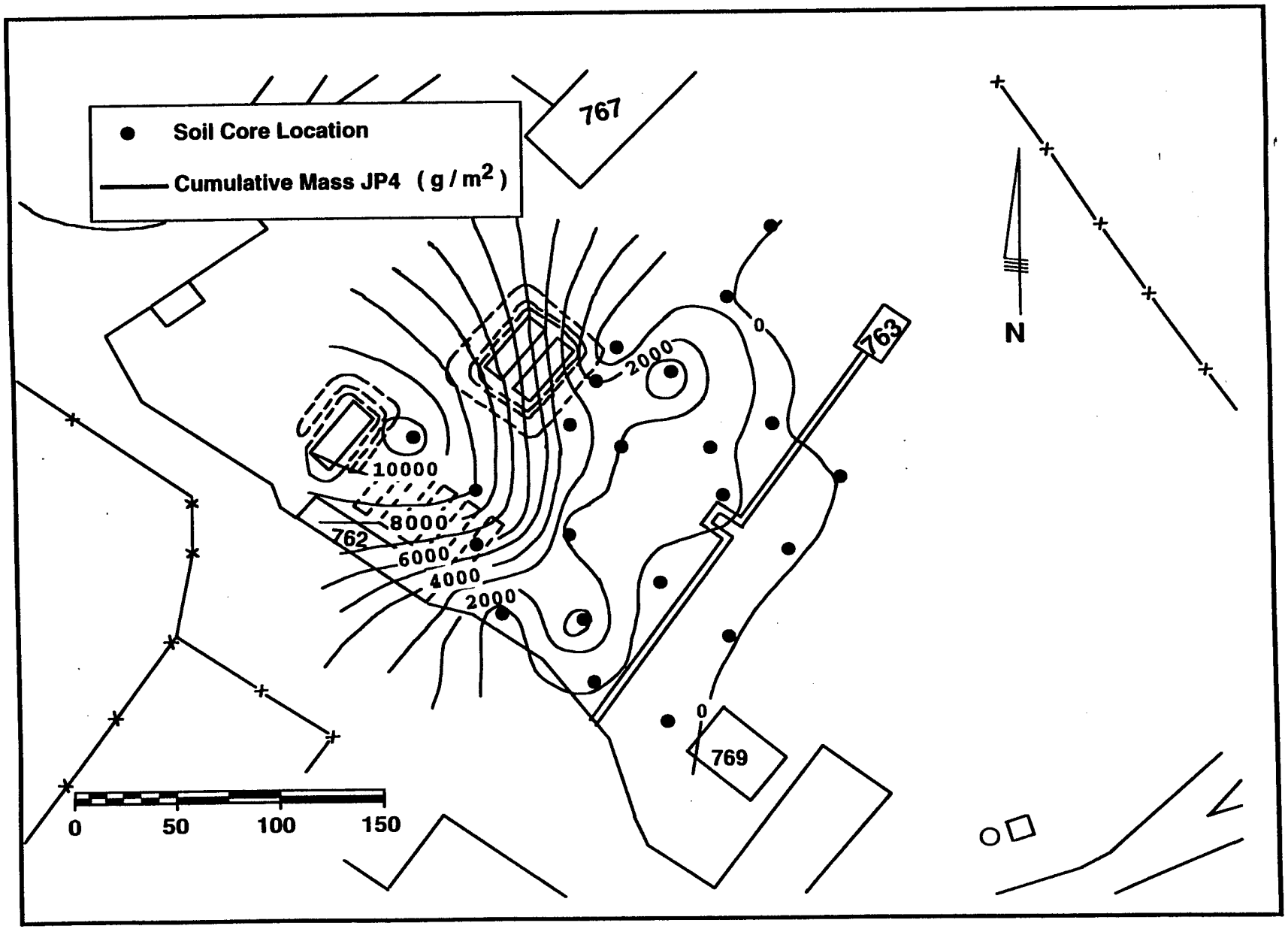


FIGURE 7. CONTOUR MAP OF THE CUMULATIVE MASS OF JP-4 PRIOR TO REMEDIATION.

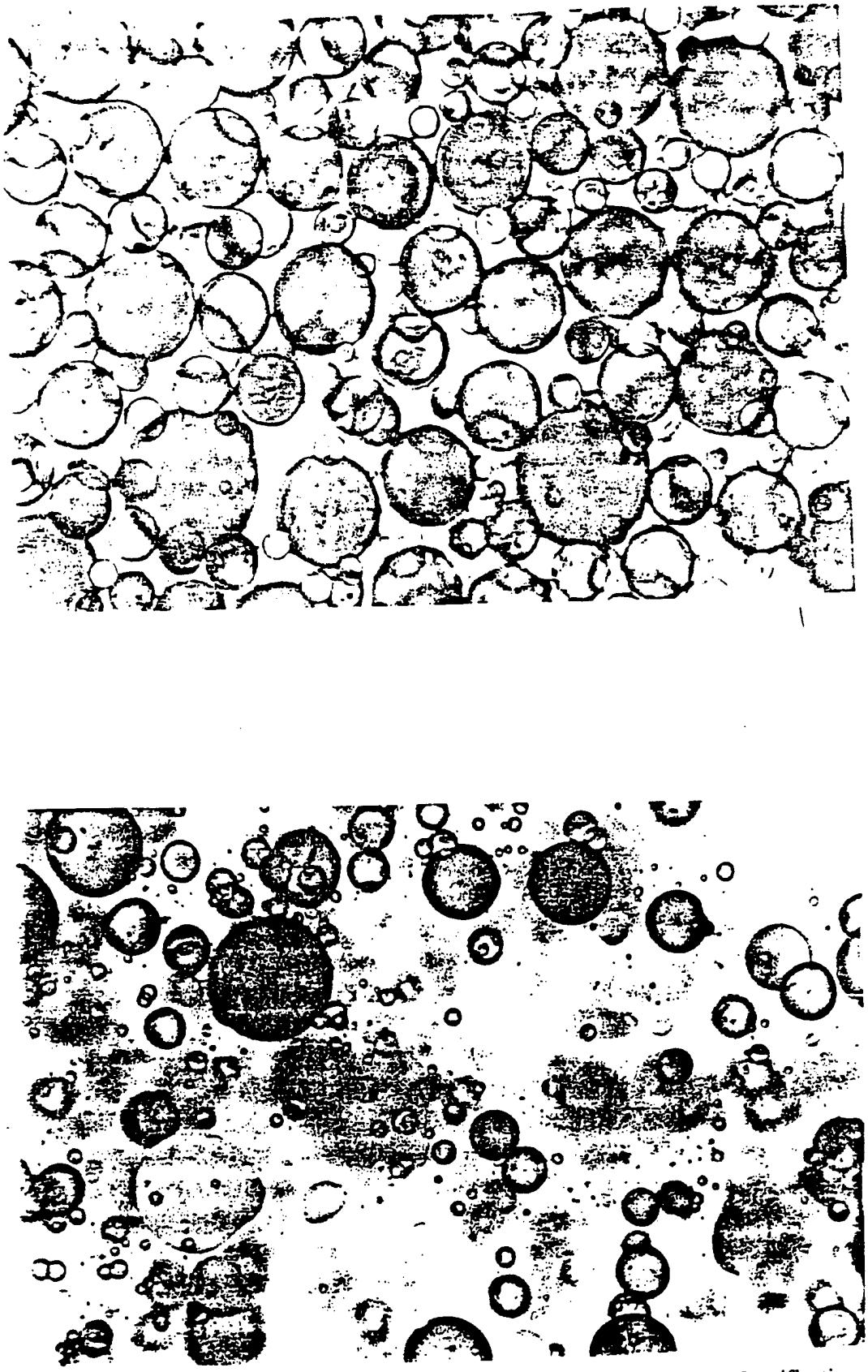


Figure 8. JP-4 and Corn Oil in Agarose (New Method--Top; Old Method--Bottom) 32 x Magnification.

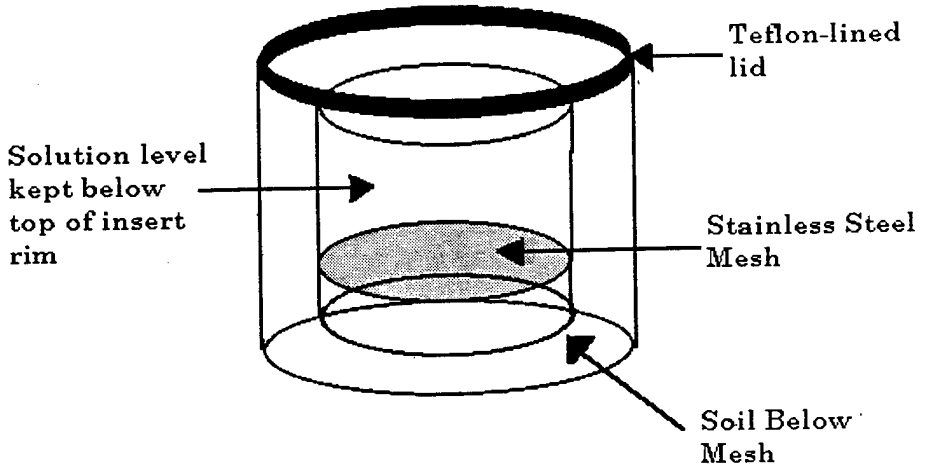


FIGURE 9. DIAGRAM OF EXPOSURE CHAMBER INSERT USED IN DIRECT EXPOSURE EXPERIMENTS.

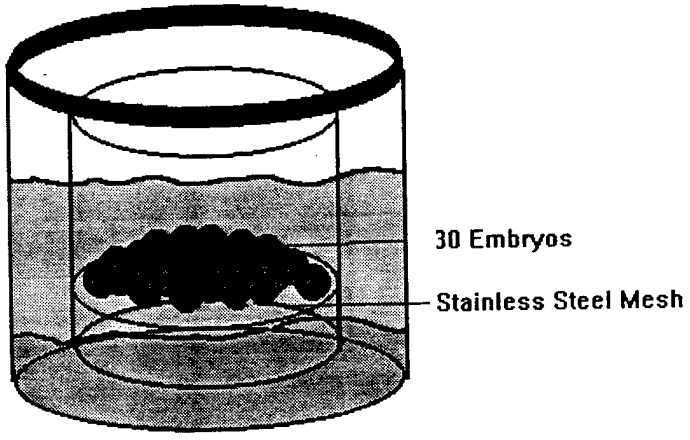


FIGURE 10. DIRECT EXPOSURE JAR WITH INSERT.

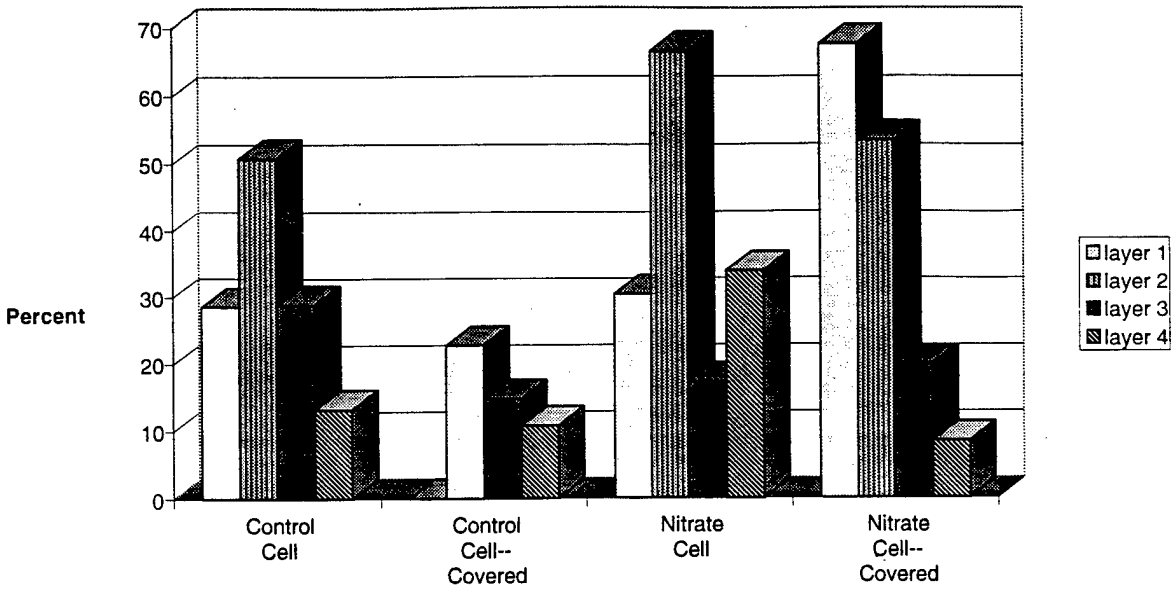


FIGURE 11. SUMMARY OF % MALFORMATION FOR ALL LAYERS OF POST REMEDIATION SOIL SAMPLES

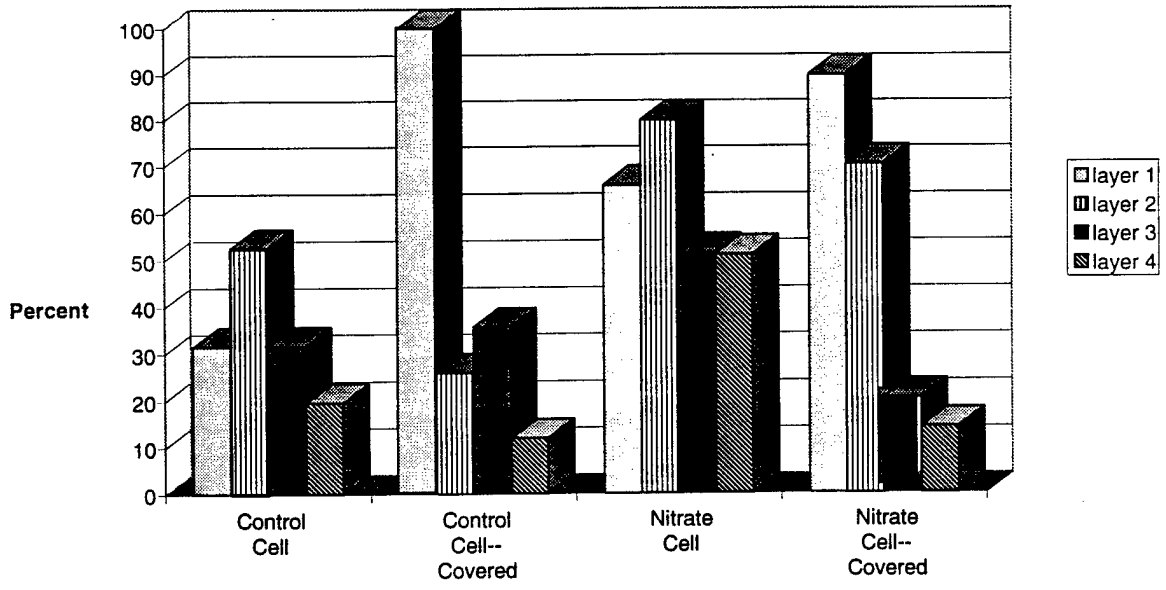


FIGURE 12. SUMMARY OF % MORTALITY FOR ALL LAYERS OF POST REMEDIATION SOIL SAMPLES

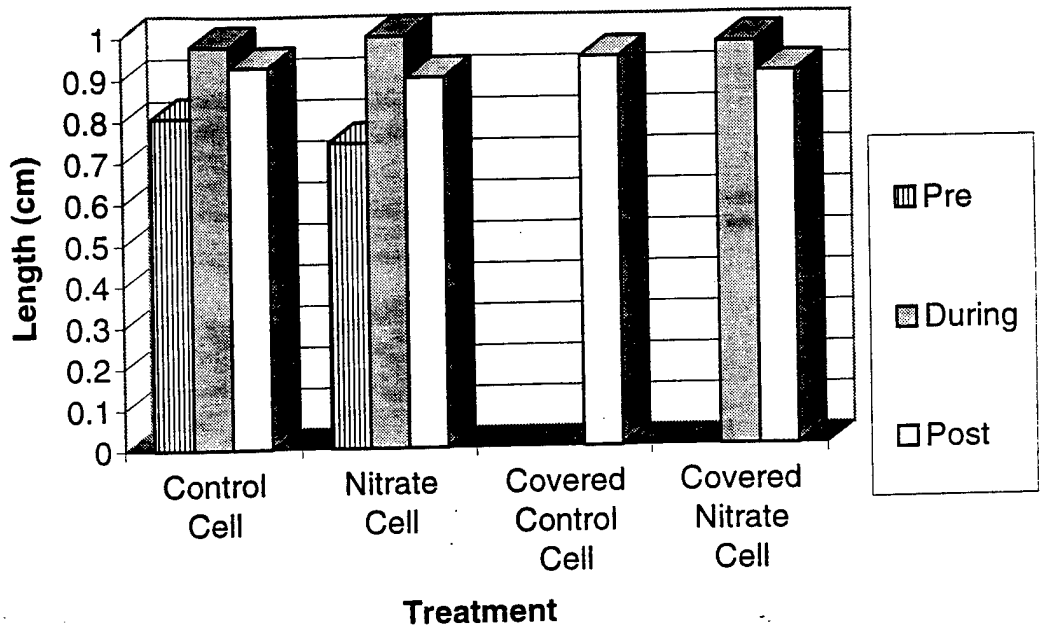


FIGURE 13. SUMMARY OF LENGTH DATA FOR EACH TREATMENT OVER THE THREE COLLECTING PERIODS. (Lack of bar indicates that this site was not tested during this time period.)

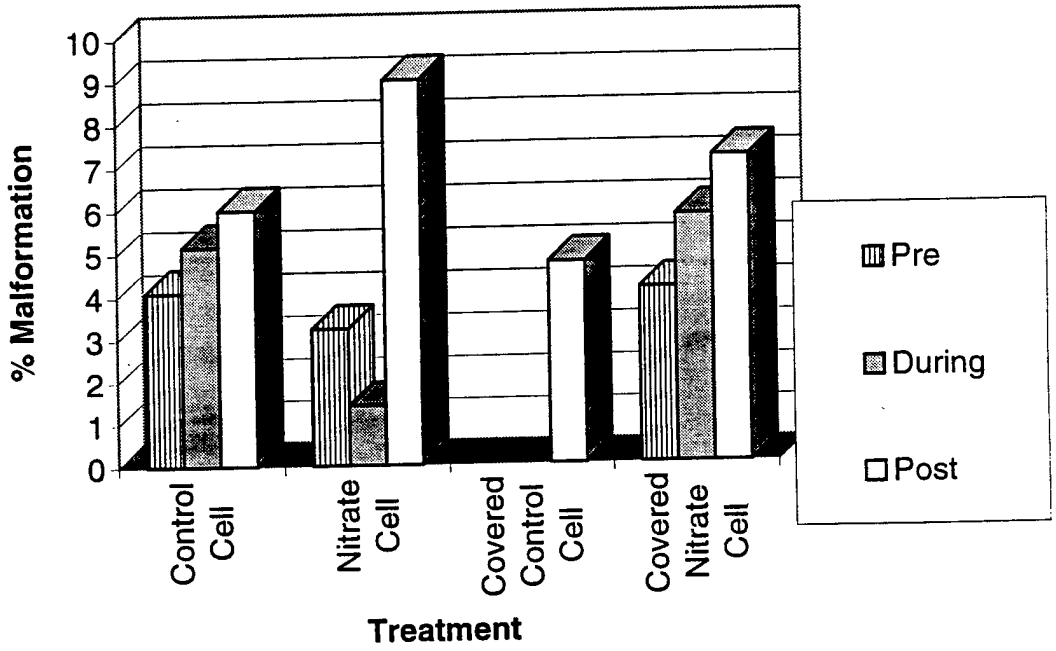


FIGURE 14. SUMMARY OF % MALFORMATION DATA FOR EACH TREATMENT OVER THE THREE COLLECTING PERIODS

(Lack of bar indicates that this site was not tested during this time period.)

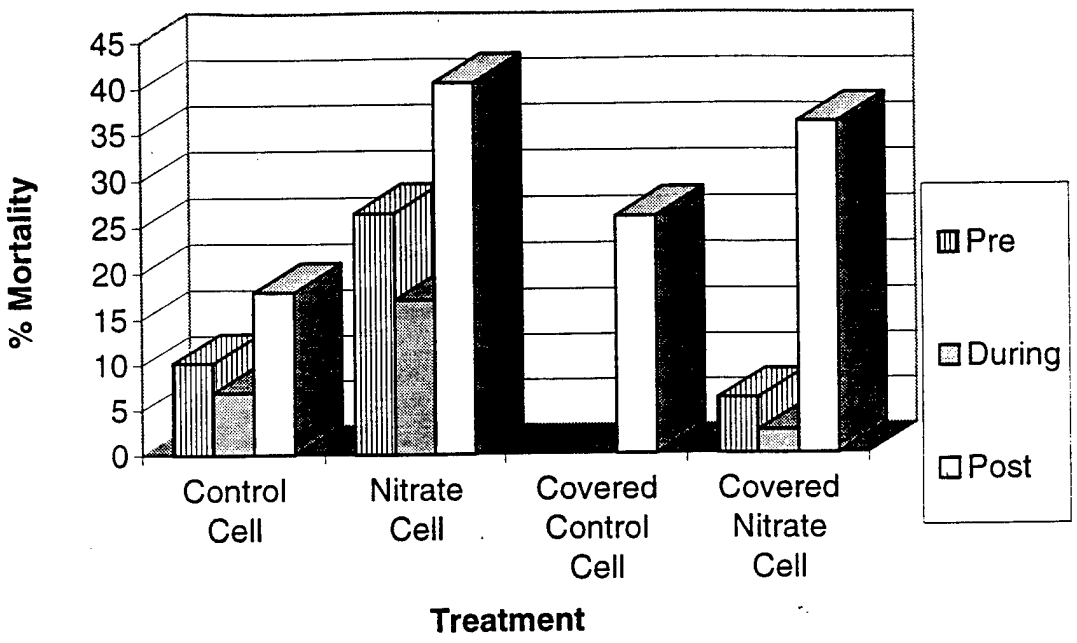


FIGURE 15. SUMMARY OF % MORTALITY DATA FOR EACH TREATMENT OVER THE THREE COLLECTING PERIODS (Lack of bar indicates that this site was not tested during this time period.)

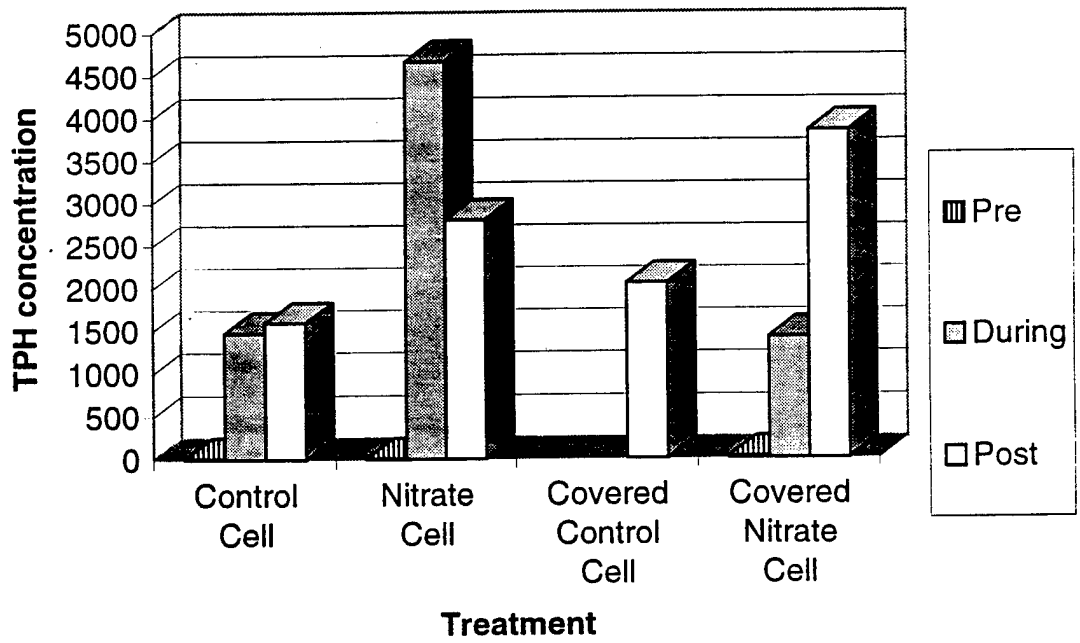


FIGURE 16. SUMMARY OF TPH VALUES FOR EACH TREATMENT OVER THE THREE COLLECTING PERIODS. (Lack of bar indicates that this site was not tested during this time period.)

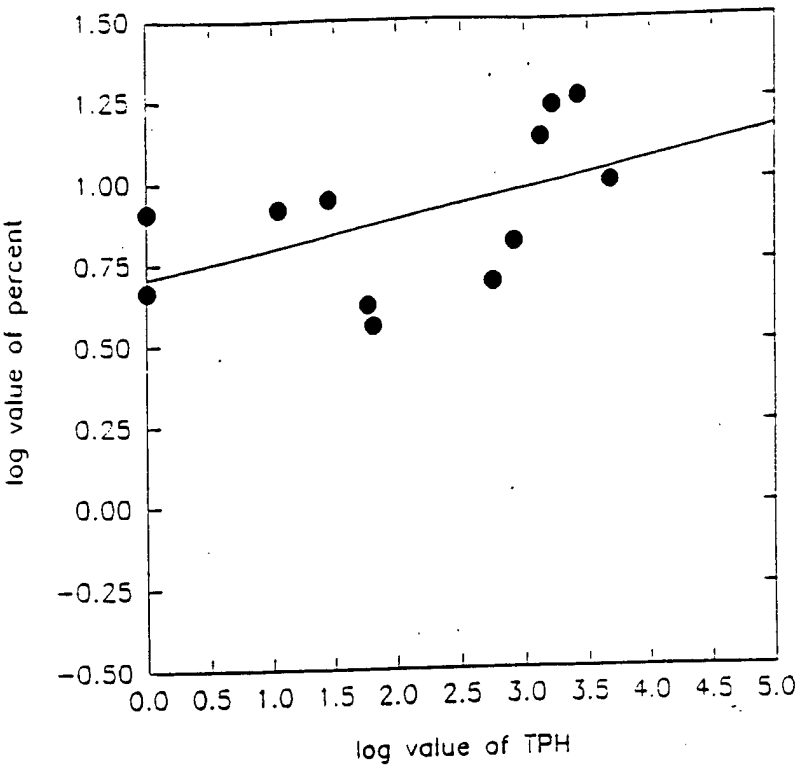


FIGURE 17. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE NITRATE-TREATED CELL, POST REMEDIATION SAMPLE.

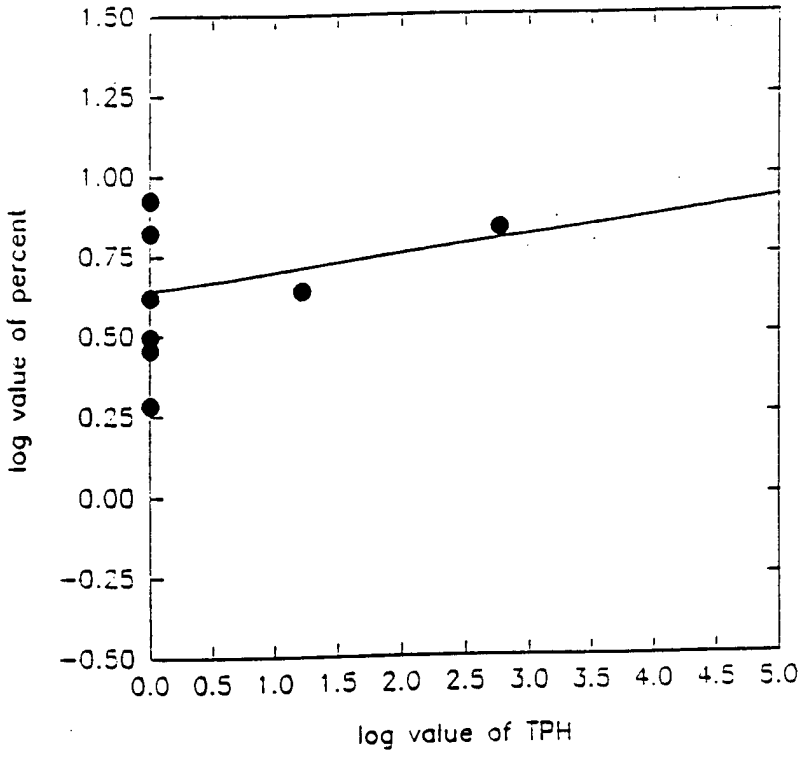


FIGURE 18. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE COVERED CONTROL CELL, POST REMEDIATION SAMPLE.

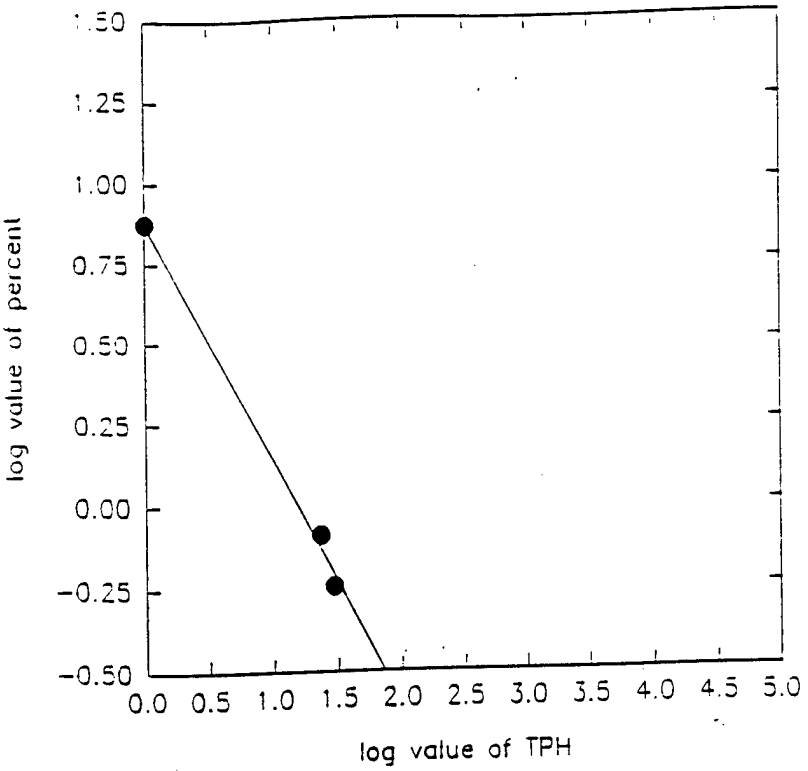


FIGURE 19. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE CONTROL CELL, PRE REMEDIATION SAMPLE.

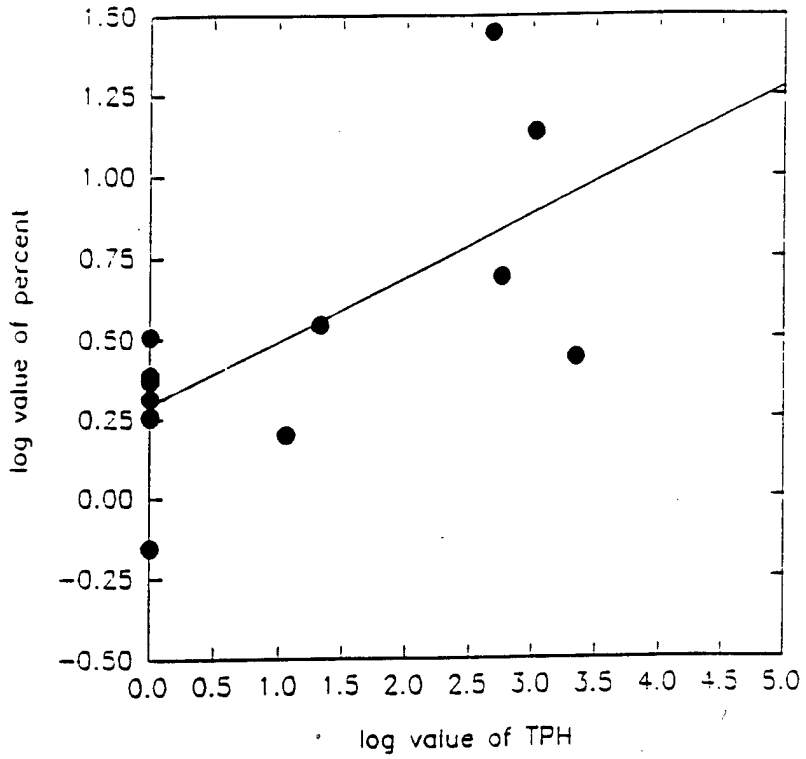


FIGURE 20. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE CONTROL CELL, DURING REMEDIATION SAMPLE.

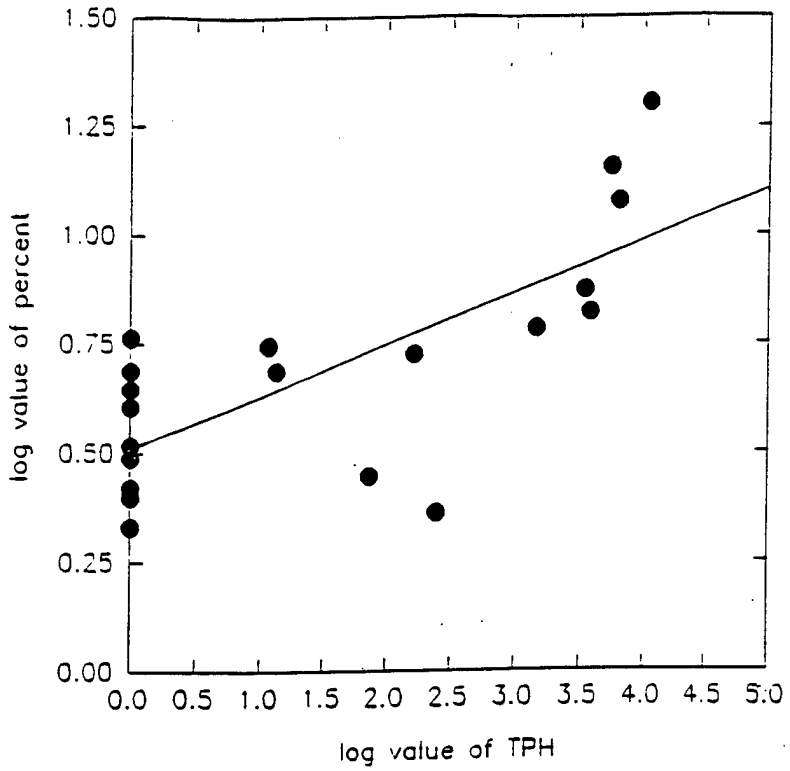


FIGURE 21. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE CONTROL CELL, POST REMEDIATION SAMPLE.

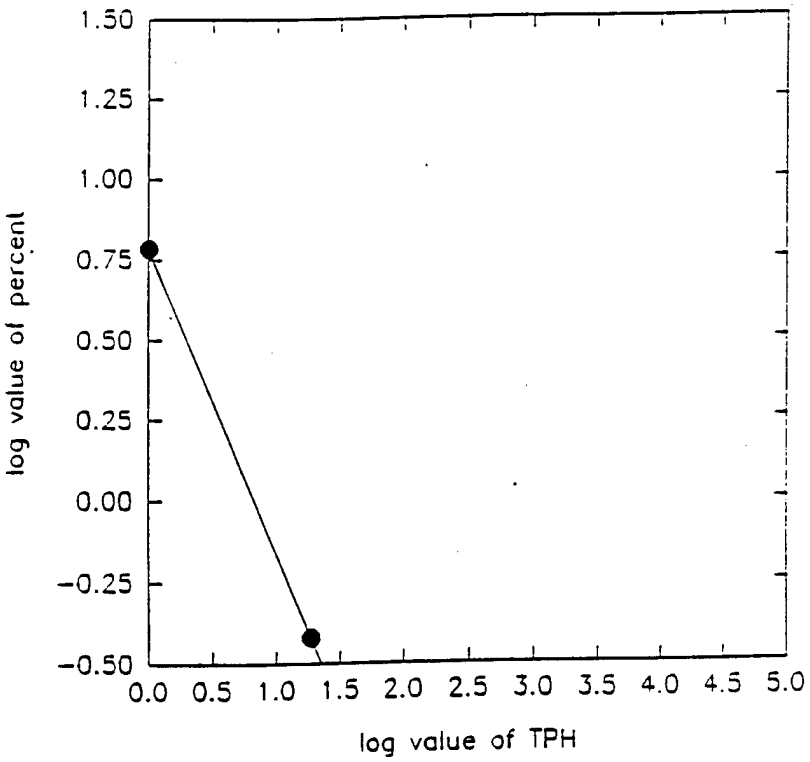


FIGURE 22. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE NITRATE CELL, PRE REMEDIATION SAMPLE.

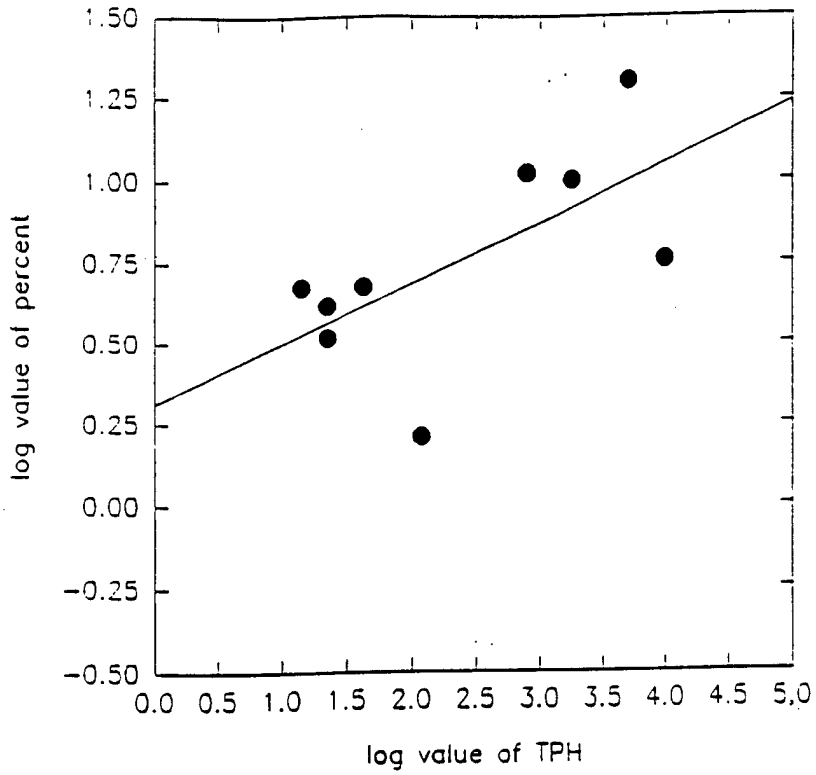


FIGURE 23. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE COVERED NITRATE CELL, POST REMEDIATION SAMPLE.

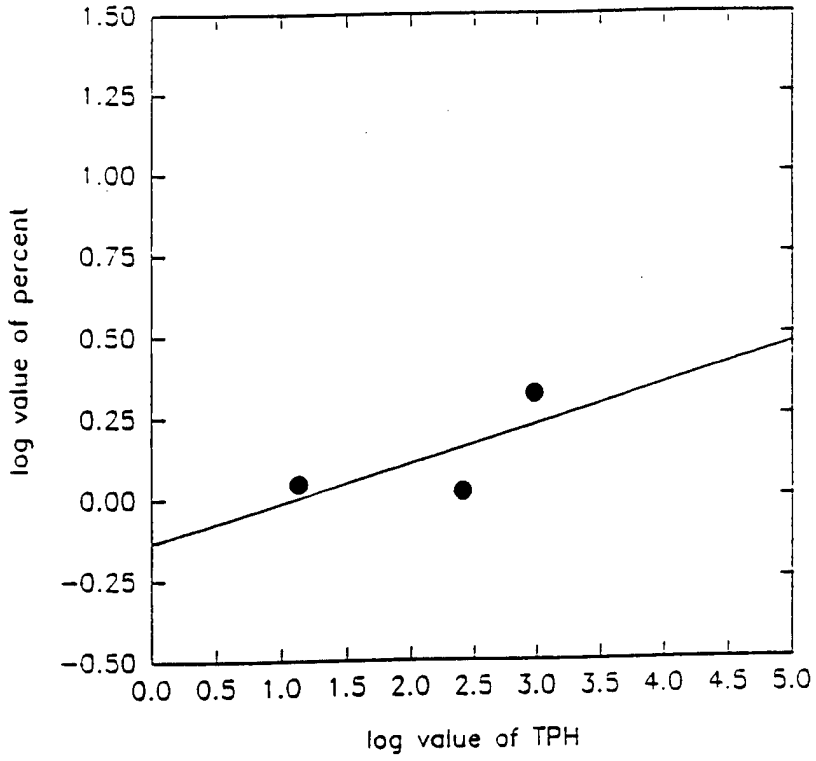


FIGURE 24. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR THE NITRATE CELL, DURING REMEDIATION SAMPLE.

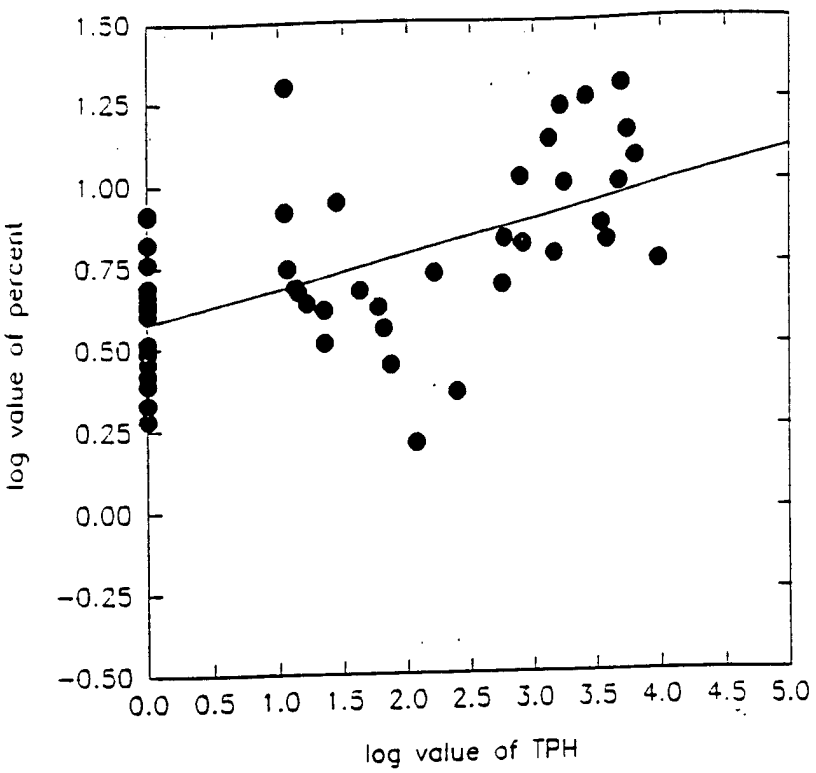


FIGURE 25. REGRESSION OF THE LOG VALUES OF MALFORMATION AND TPH FOR POST REMEDIATION SAMPLE.

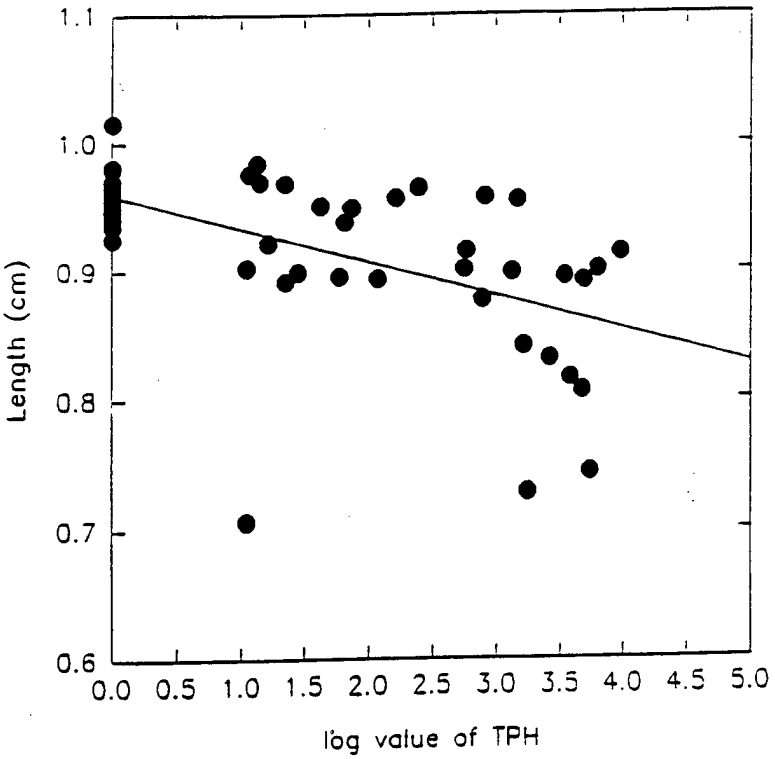


FIGURE 26. REGRESSION OF THE LENGTH AND LOG VALUES OF TPH FOR POST REMEDIATION SAMPLE.

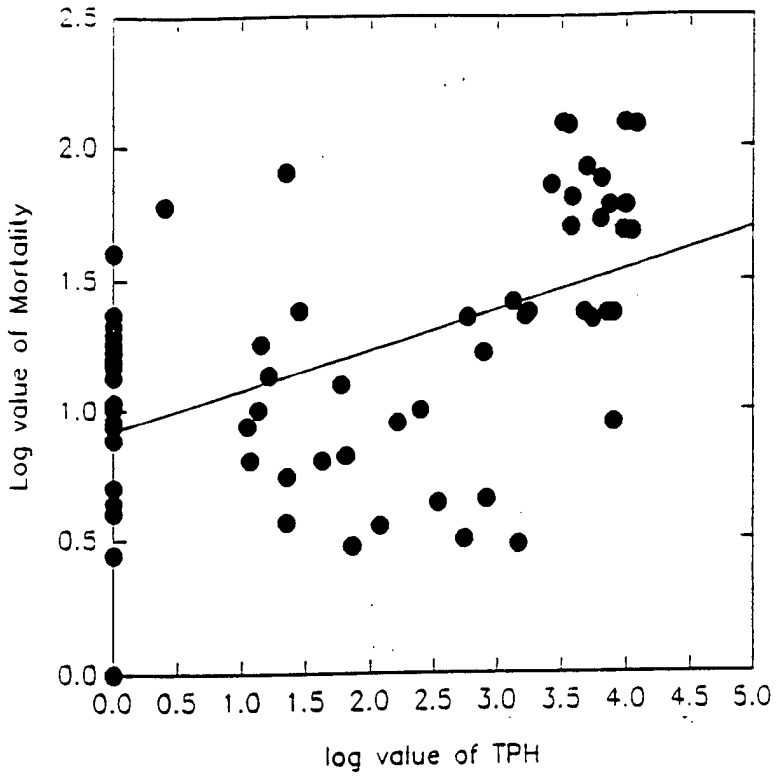


FIGURE 27. REGRESSION OF THE LOG VALUES OF MORTALITY AND TPH FOR POST REMEDIATION SAMPLE.

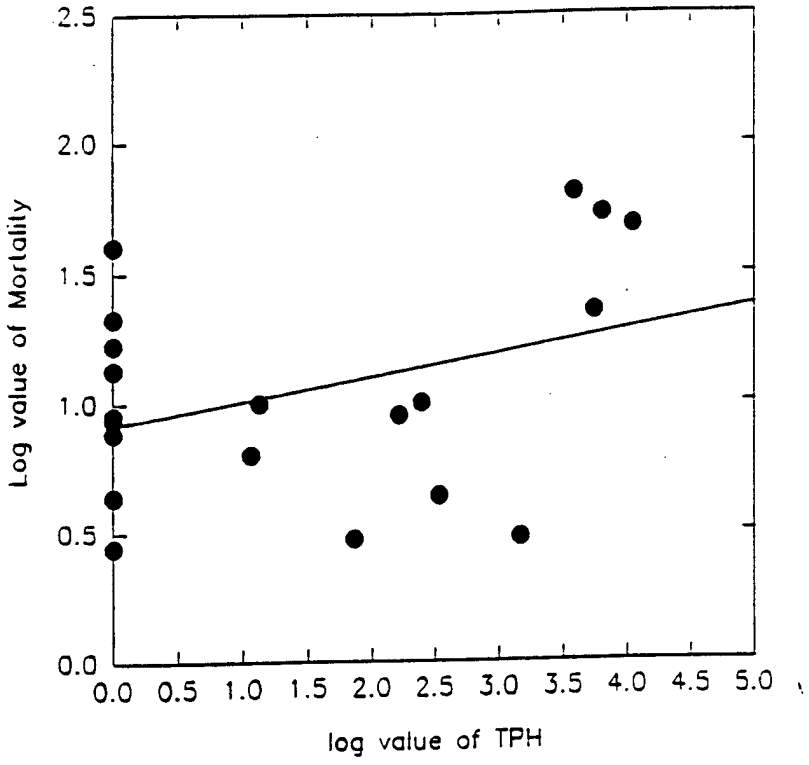


FIGURE 28. REGRESSION OF THE LOG VALUES OF PERCENT MORTALITY AND TPH FOR POST REMEDIATION SAMPLE IN THE CONTROL CELL.

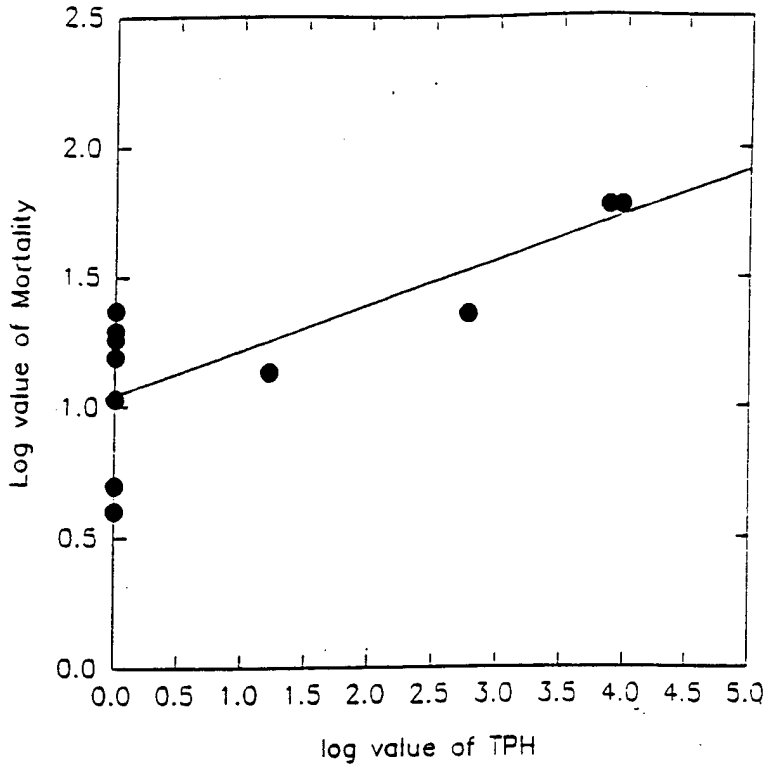


FIGURE 29. REGRESSION OF THE LOG VALUES OF MORTALITY AND TPH FOR POST REMEDIATION SAMPLE FROM THE COVERED CONTROL CELL.

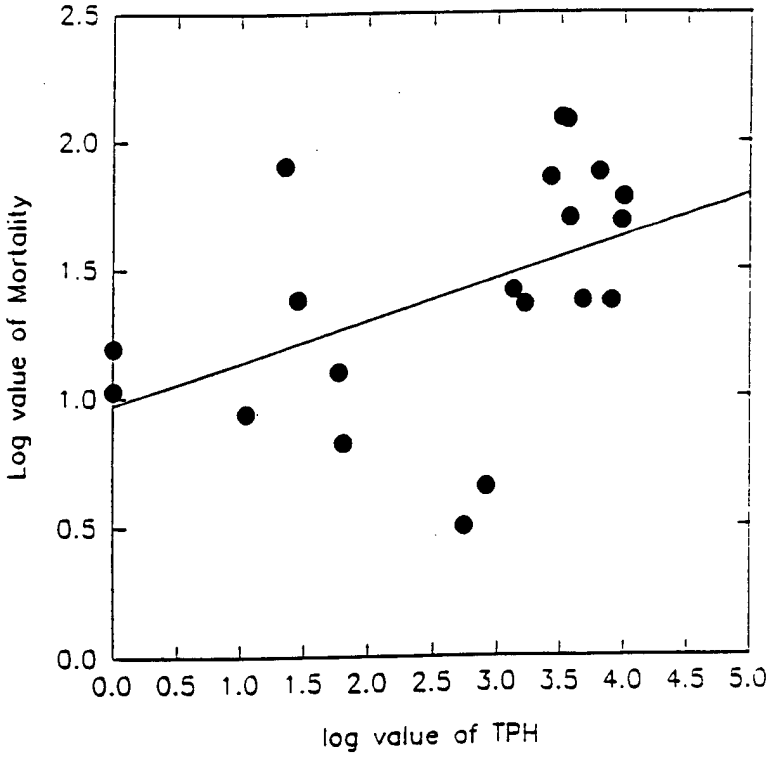


FIGURE 30. REGRESSION OF THE LOG VALUES OF MORTALITY AND TPH FOR POST REMEDIATION SAMPLE FROM THE NITRATE CELL.

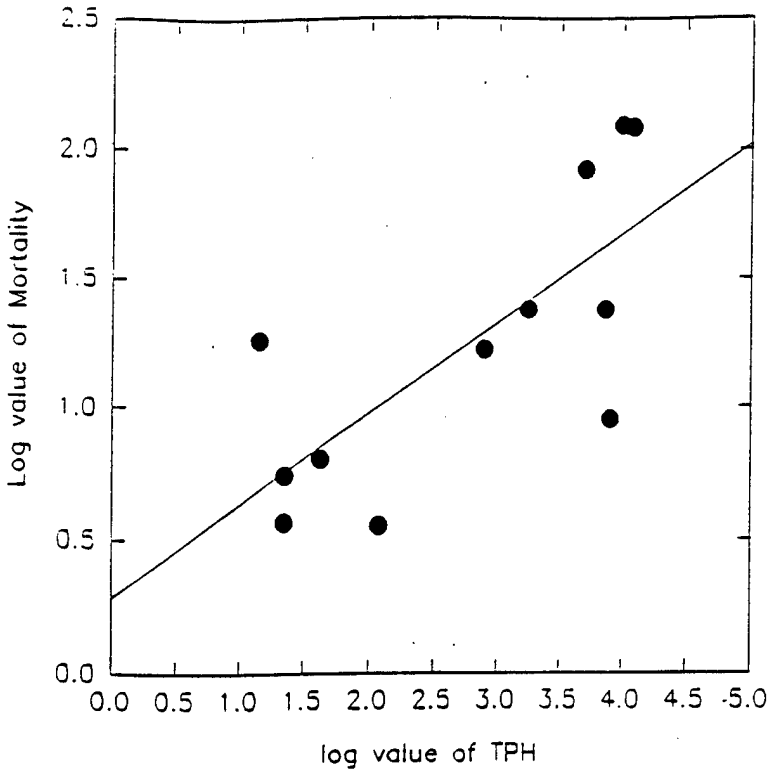


FIGURE 31. REGRESSION OF THE LOG VALUES OF MORTALITY AND TPH FOR POST REMEDIATION SAMPLE FROM THE COVERED NITRATE CELL.

Pre Remediation FETAX Results

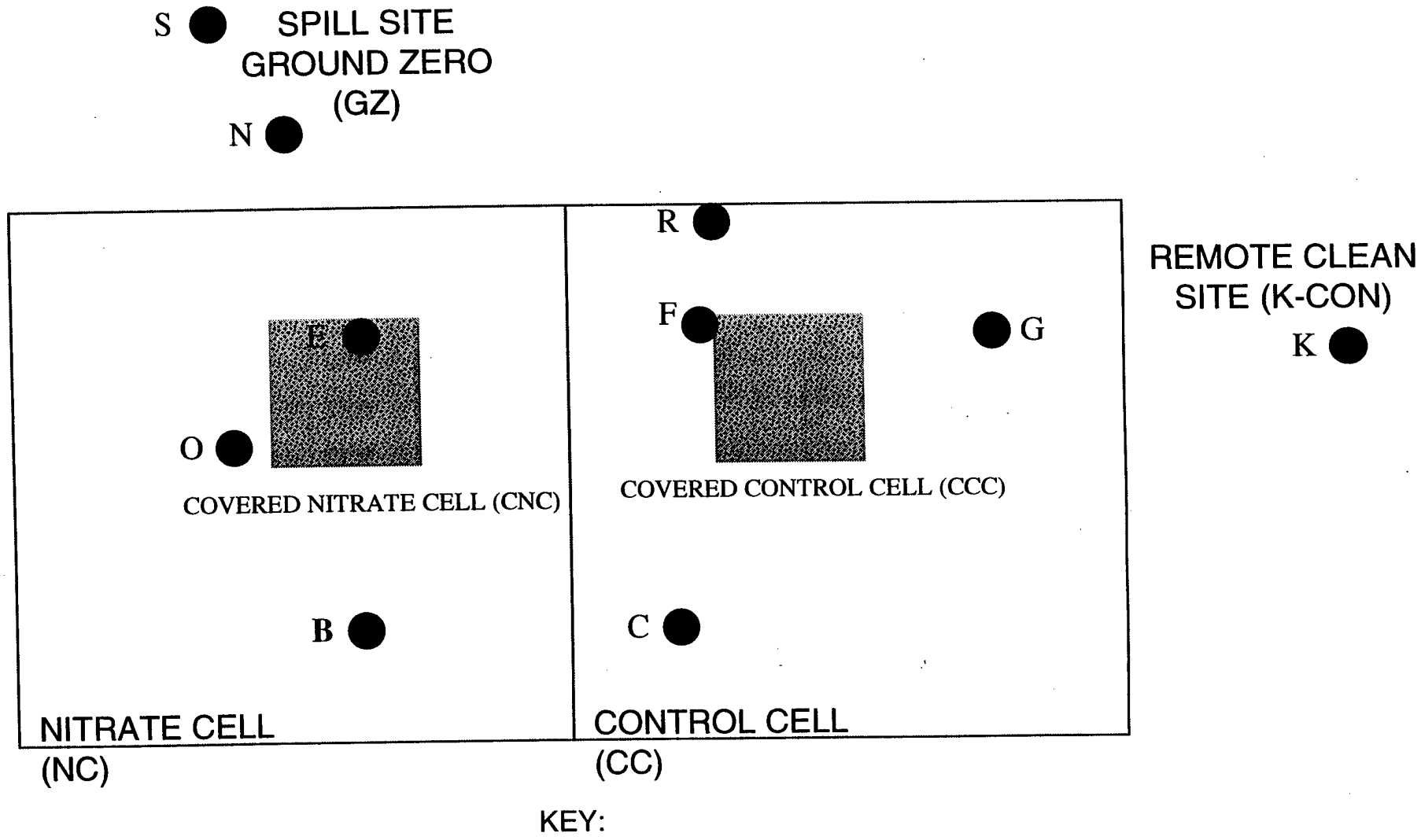


FIGURE 32. LOCATION OF TREATMENT CELLS AND PRE REMEDIATION COLLECTION SITES.

During Remediation FETAX Results

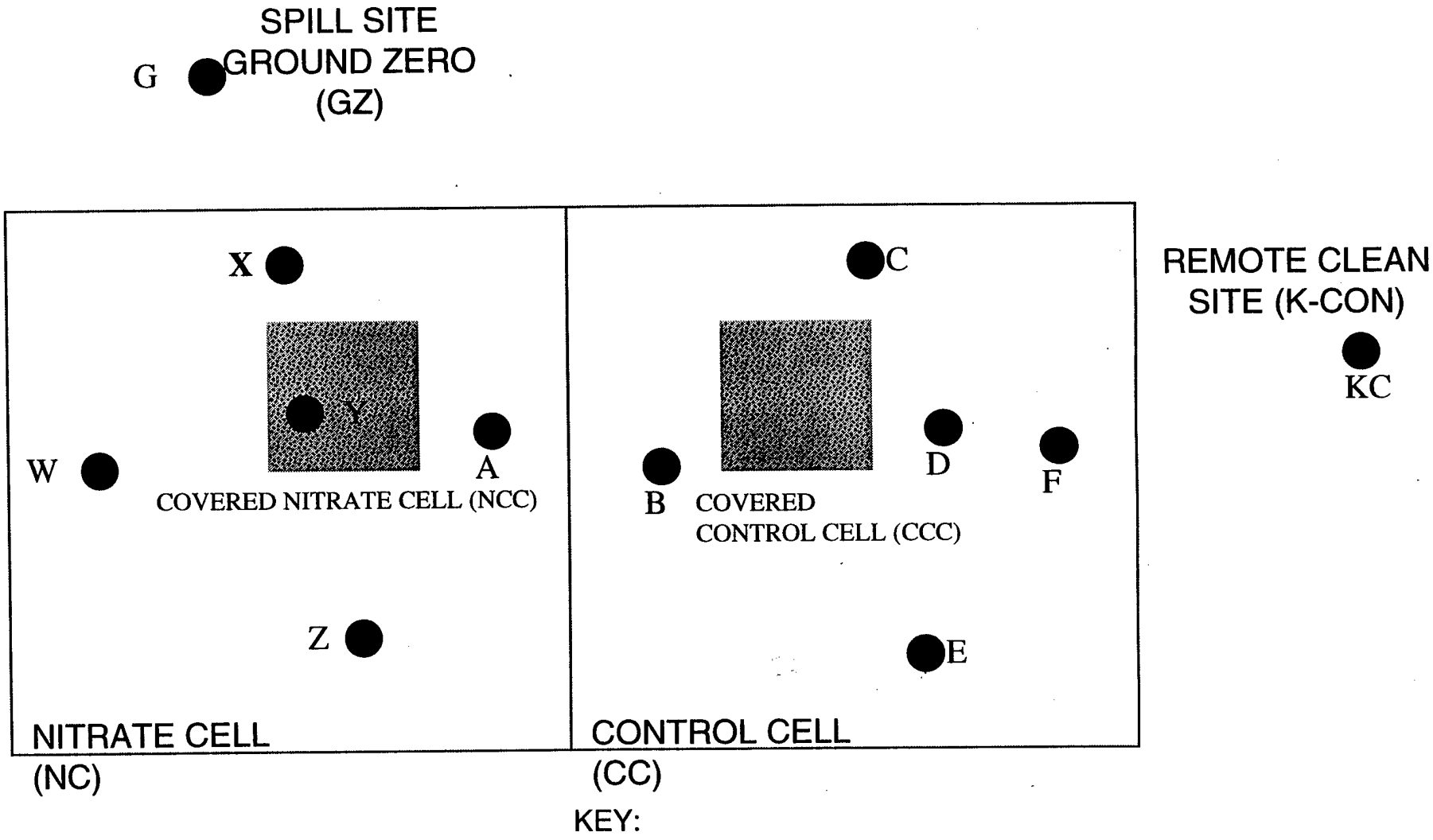


FIGURE 33. LOCATION OF TREATMENT CELLS AND DURING REMEDIATION COLLECTION SITES.

Post Remediation FETAX Results

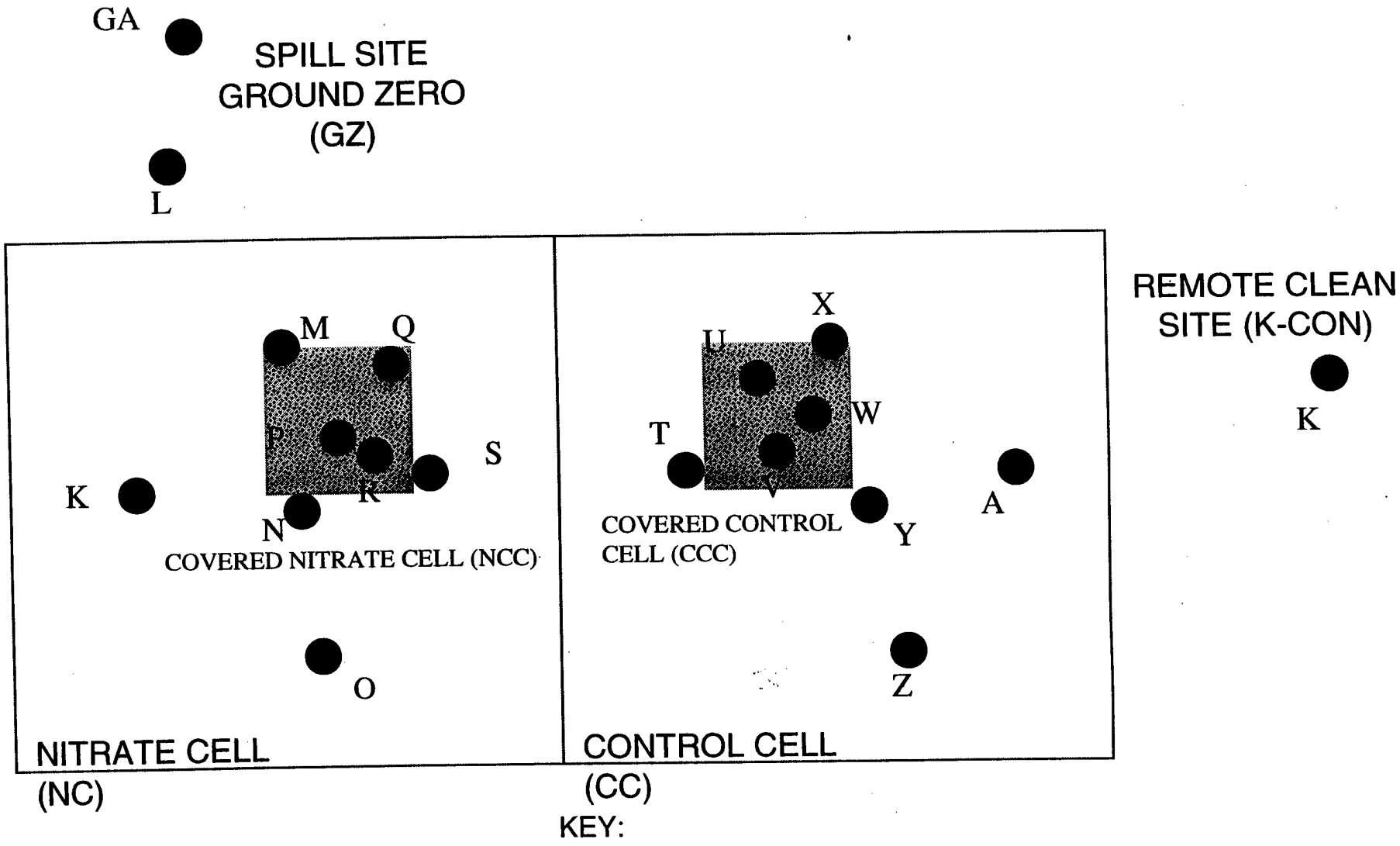
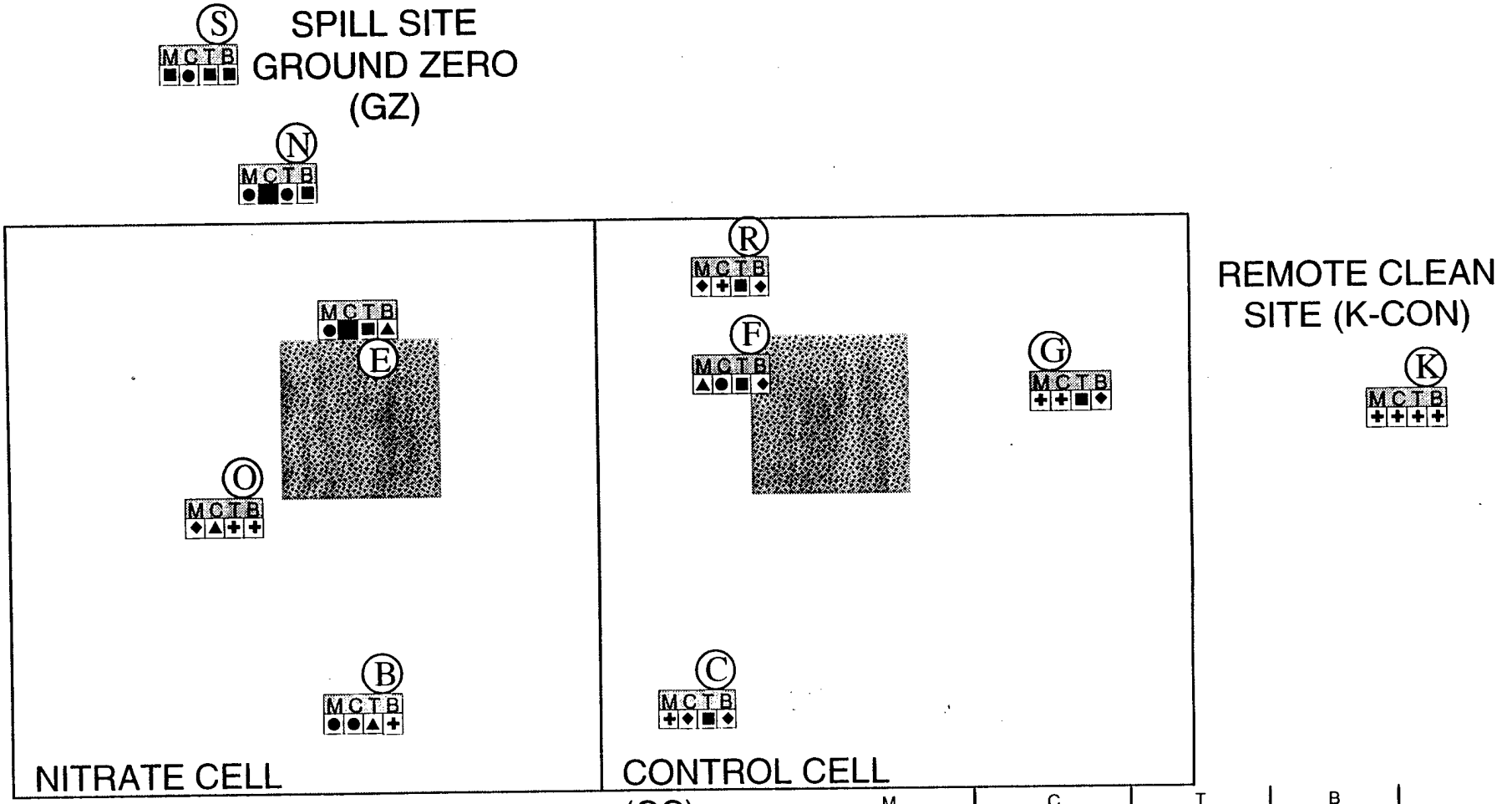


FIGURE 34. LOCATION OF TREATMENT CELLS AND POST REMEDIATION COLLECTION SITES.

Pre Remediation Results

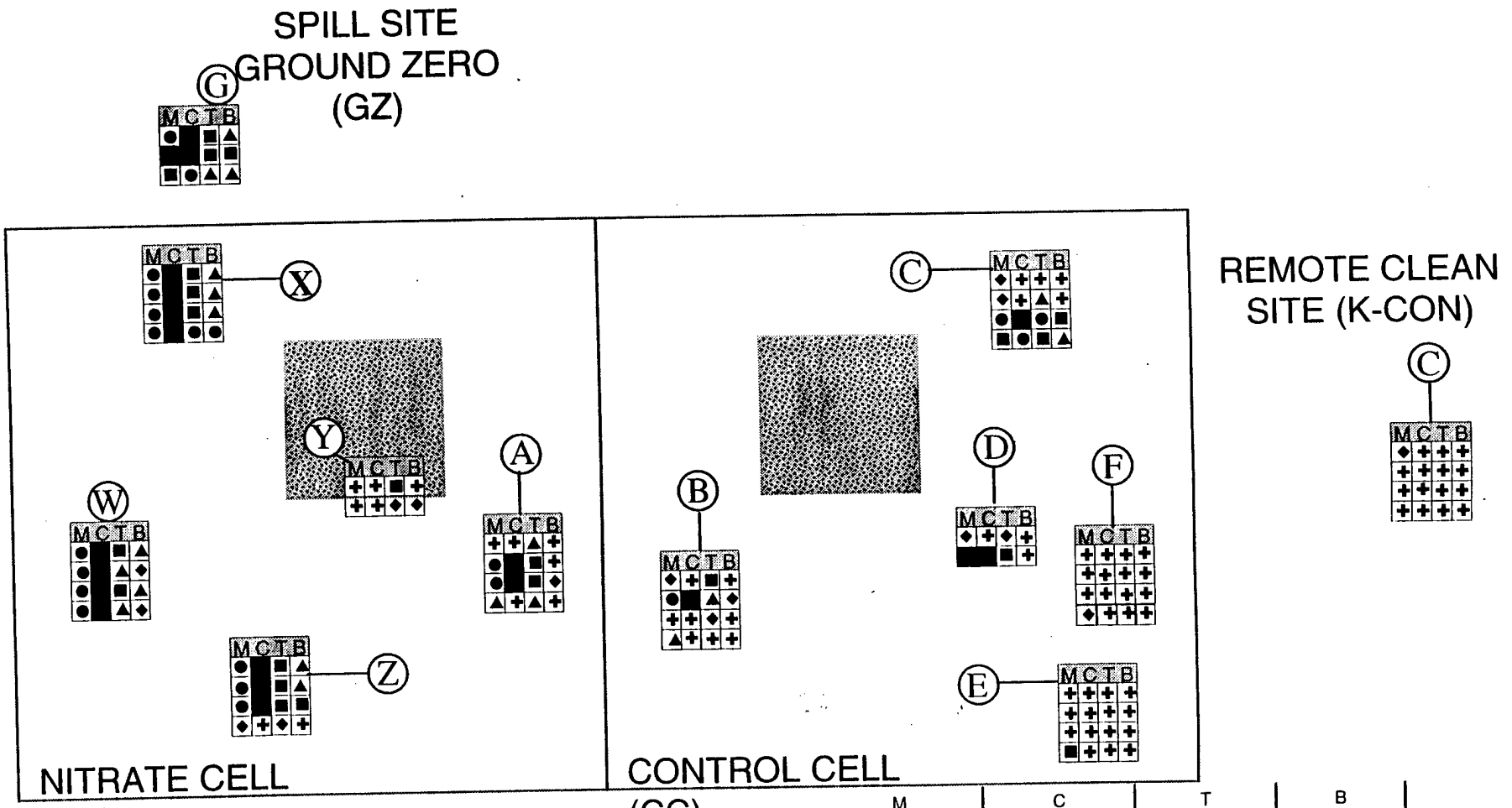


KEY:

M % Mortality	C % Malformation	T (TPH) mg/kg	B (BTEX) mg/kg	Symbol
100	100	10000	1000	●
75-99.9	75-99.9	1000-9999.9	100-999.9	■
50-74.9	50-74.9	100-999.9	10-99.9	▲
25-49.9	25-49.9	10-99.9	1-9.9	◆
0-24.9	0-24.9	0-9.9	1-0.9	+

FIGURE 35. MORTALITY, MALFORMATION, TPH, AND BTEX DATA FOR PRE REMEDIATION SITES.

During Remediation Results

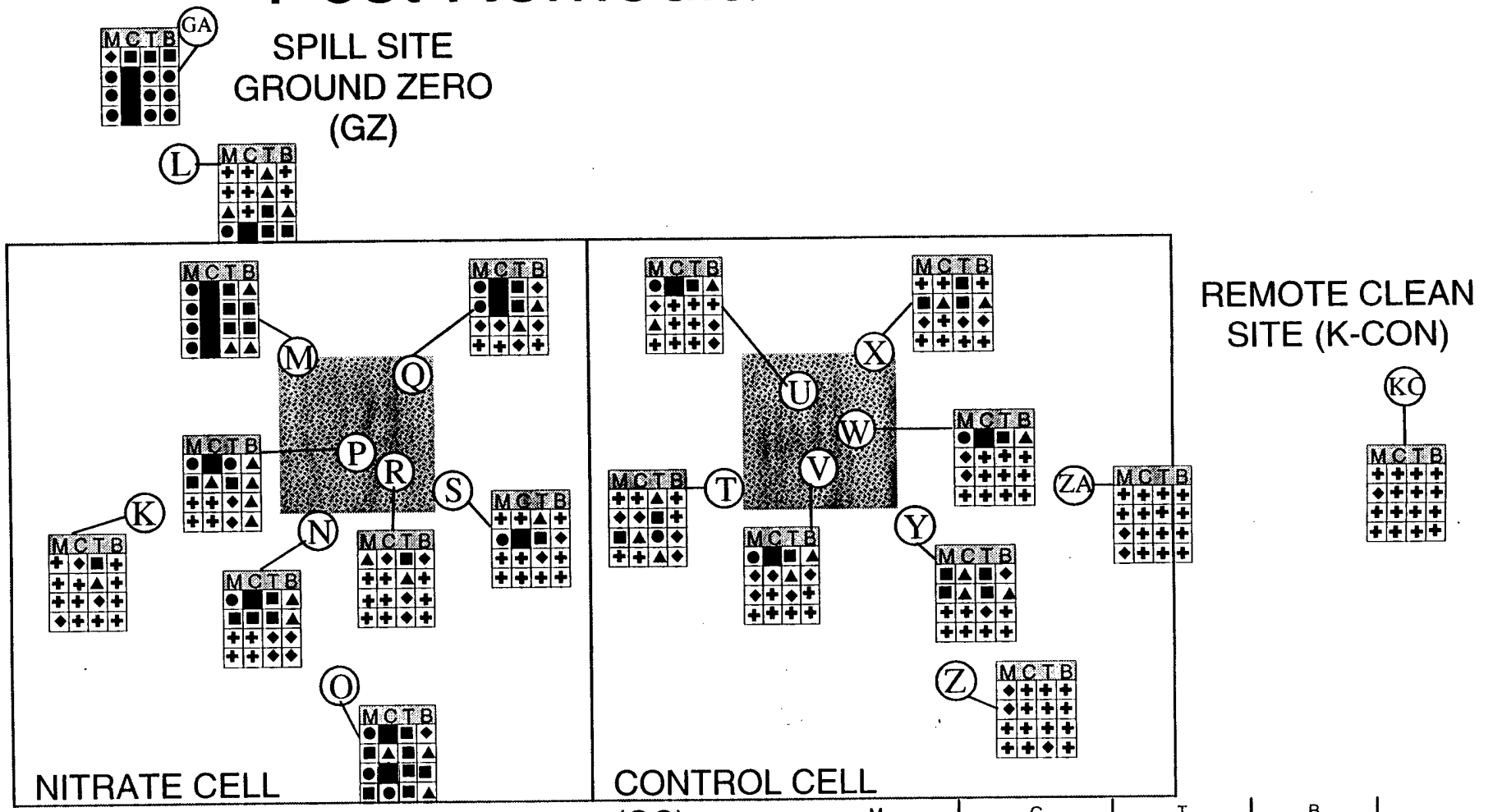


KEY:

M % Mortality	C % Malformation	T (TPH) mg/kg	B (BTEX) mg/kg	Symbol
100	100	10000	1000	●
75-99.9	75-99.9	1000-9999.9	100-999.9	■
50-74.9	50-74.9	100-999.9	10-99.9	▲
25-49.9	25-49.9	10-99.9	1-9.9	◆
0-24.9	0-24.9	0-9.9	1-0.9	✚

FIGURE 36. MORTALITY, MALFORMATION, TPH, AND BTEX DATA FOR DURING REMEDIATION SITES.

Post Remediation Results



KEY:

M % Mortality	C % Malformation	T (TPH) mg/kg	B (BTEX) mg/kg	Symbol
100	100	10000	1000	●
75-99.9	75-99.9	1000-9999.9	100-999.9	■
50-74.9	50-74.9	100-999.9	10-99.9	▲
25-49.9	25-49.9	10-99.9	1-9.9	◆
0-24.9	0-24.9	0-9.9	1-0.9	✚

FIGURE 37. MORTALITY, MALFORMATION, TPH, AND BTEX DATA FOR POST REMEDIATION SITES.

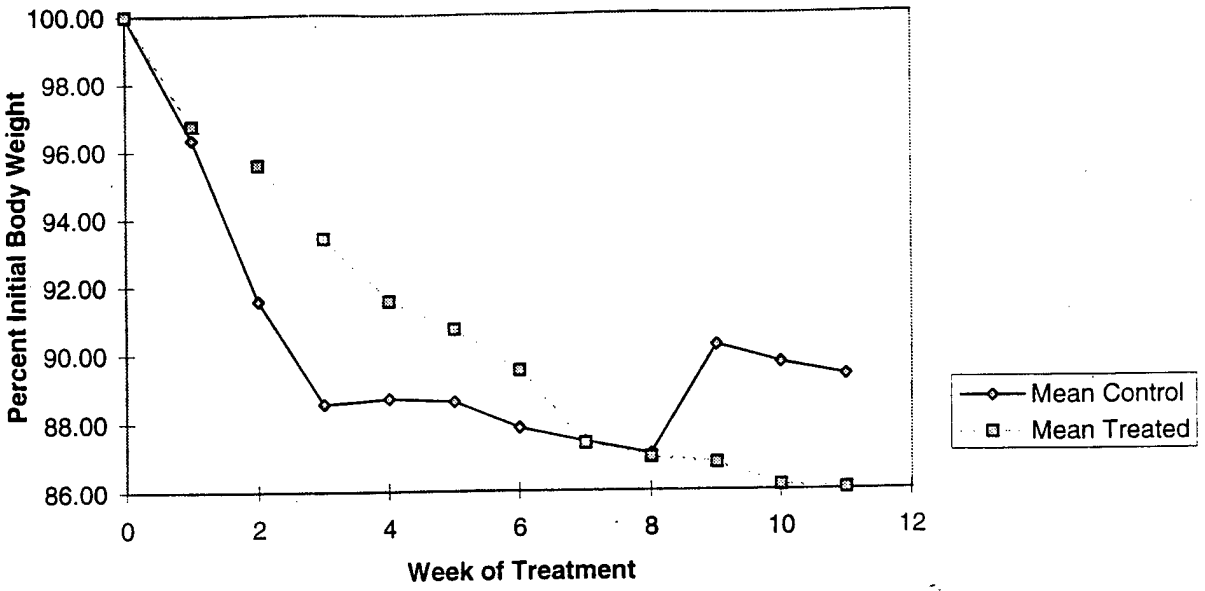


FIGURE 38. JP4 FEEDING EXPERIMENT PRE REMEDIATION BODY WEIGHT DATA

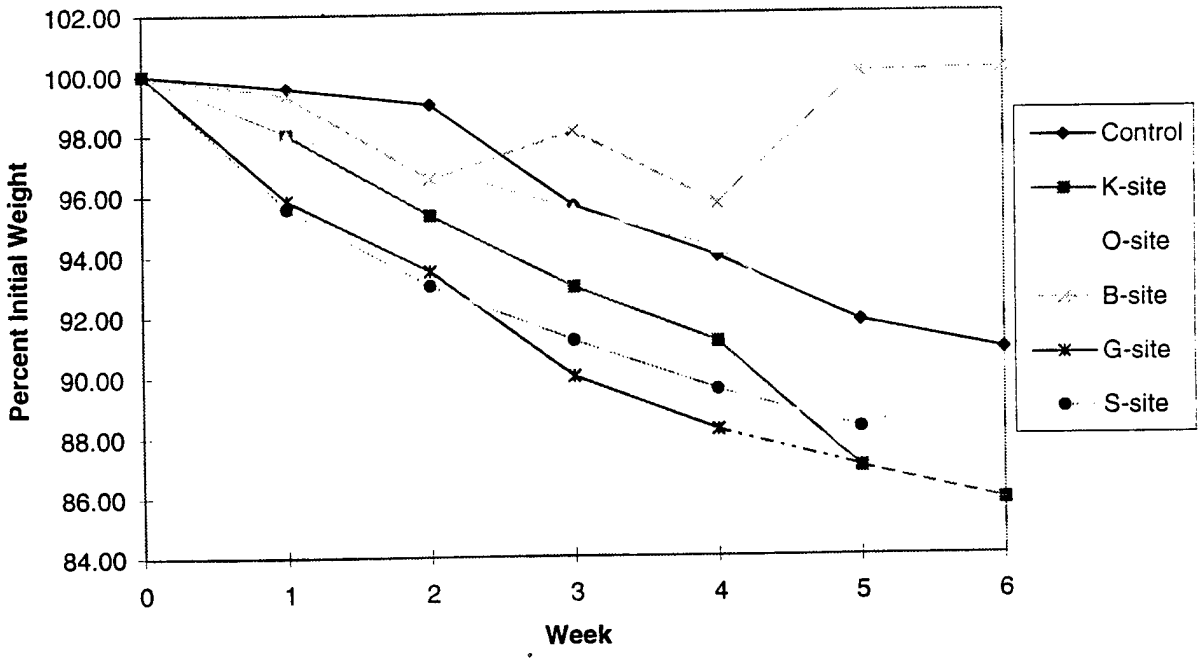


FIGURE 39. DIRECT SOIL EXPOSURE #1 PRE REMEDIATION BODY WEIGHT DATA

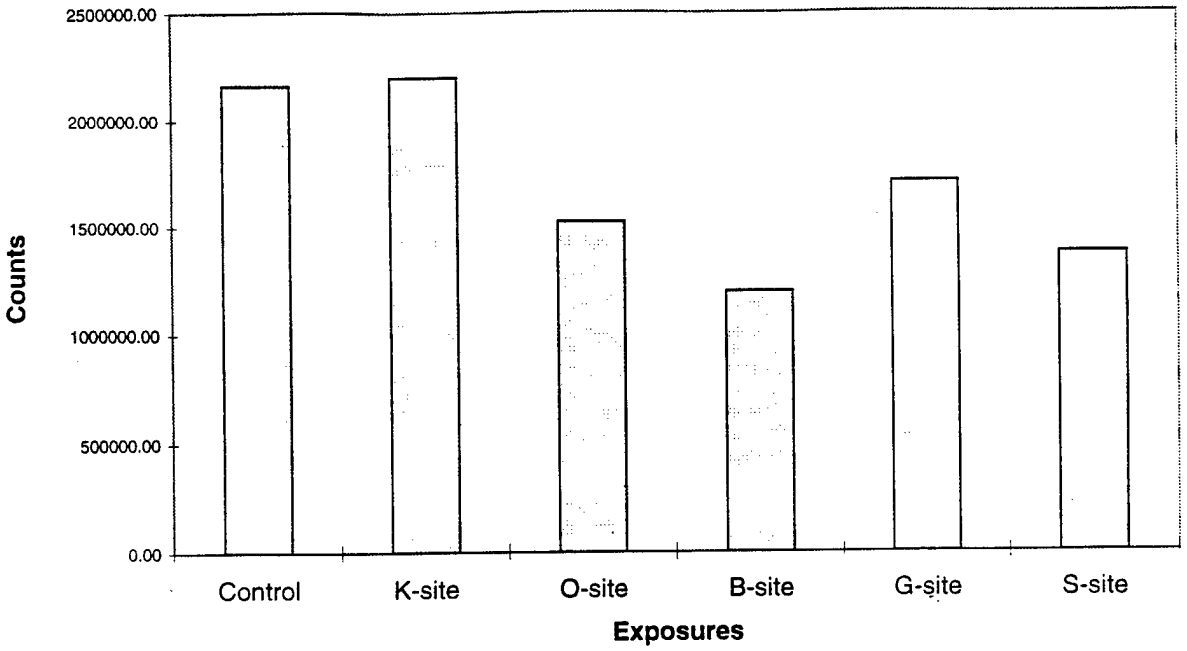


FIGURE 40. DIRECT SOIL EXPOSURE #1
PRE REMEDIATION SPERM COUNT DATA

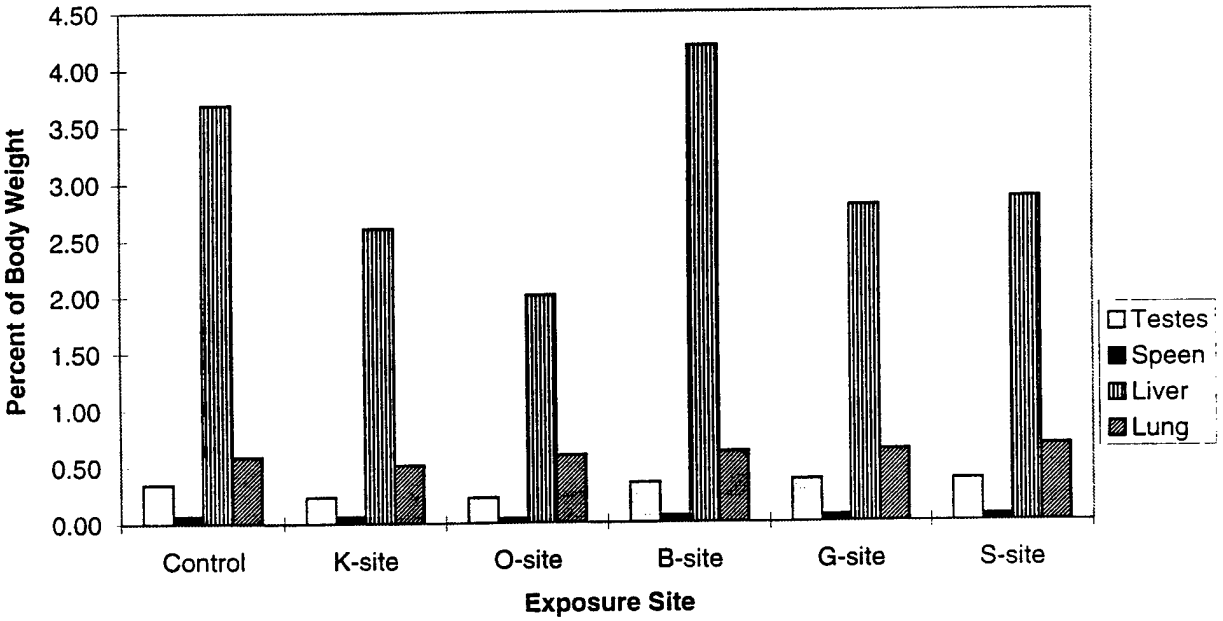


FIGURE 41. DIRECT SOIL EXPOSURE #1
PRE REMEDIATION ORGAN WEIGHT DATA

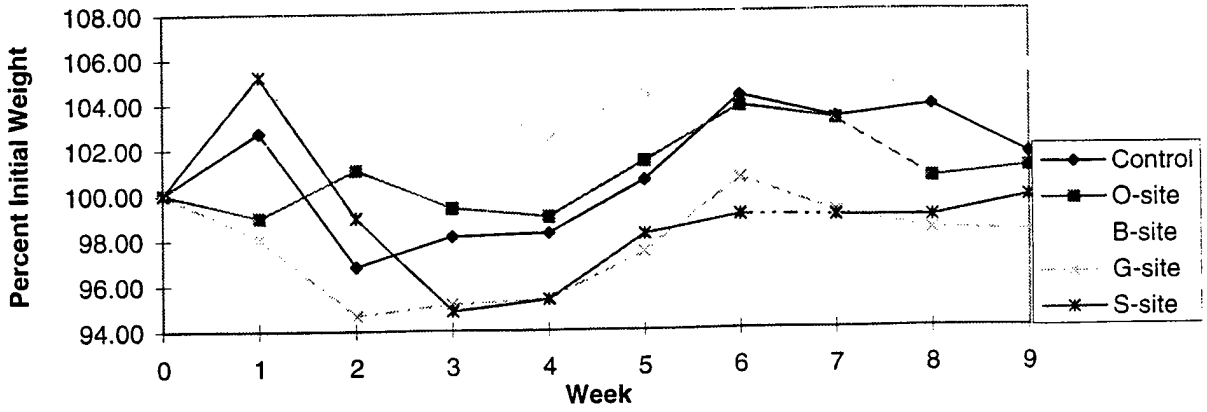


FIGURE 42. SCFE ORAL EXPOSURE #2
PRE REMEDIATION BODY WEIGHT DATA

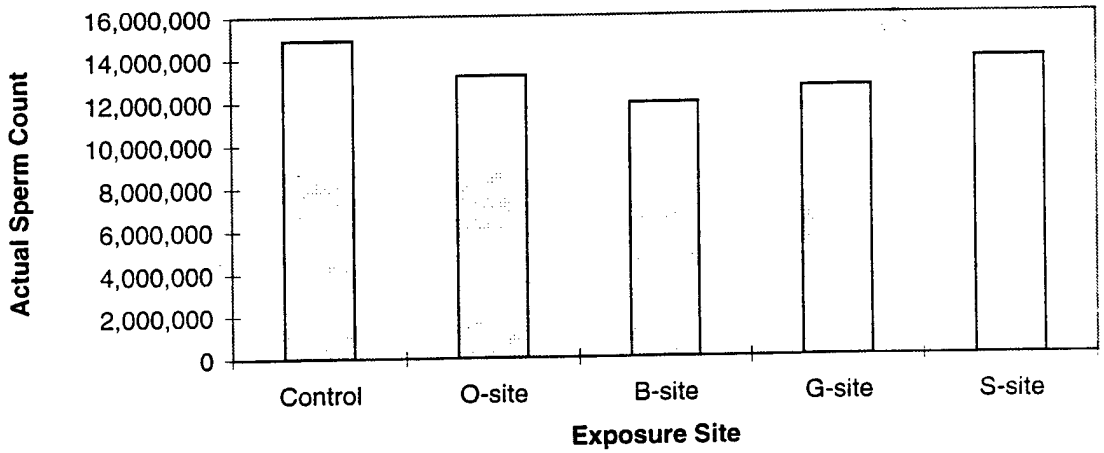


FIGURE 43. SCFE ORAL EXPOSURE #2
PRE REMEDIATION SPERM COUNT DATA

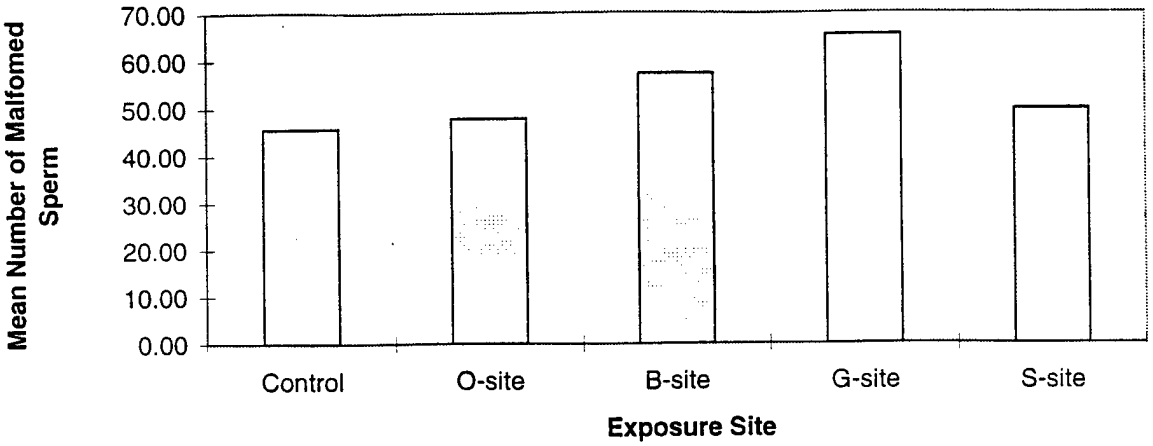


FIGURE 44. SCFE ORAL EXPOSURE #2 PRE REMEDIATION SPERM MALFORMATION DATA

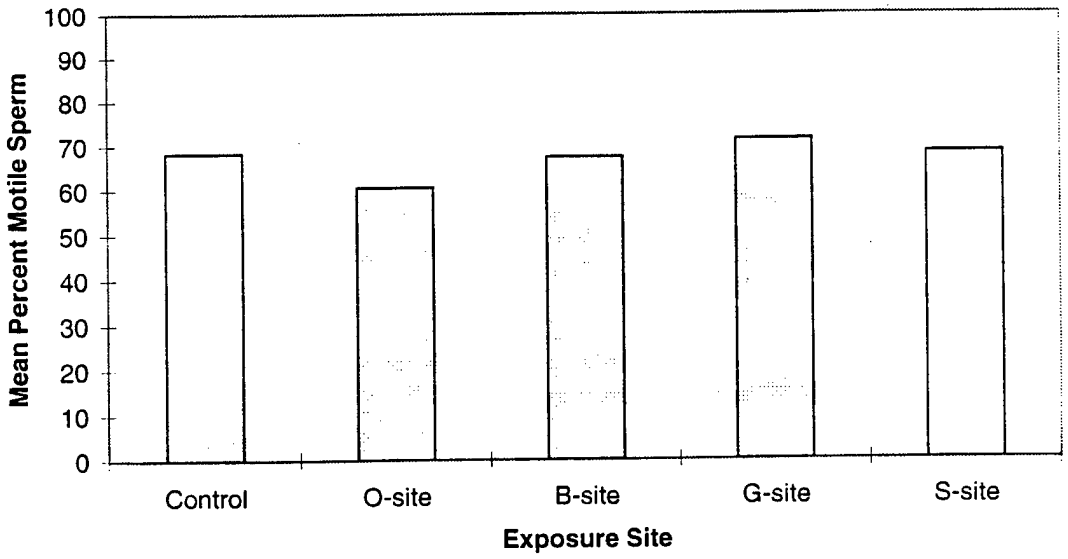


Figure 45. SCFE ORAL EXPOSURE #2 PRE REMEDIATION SPERM MOTILITY DATA

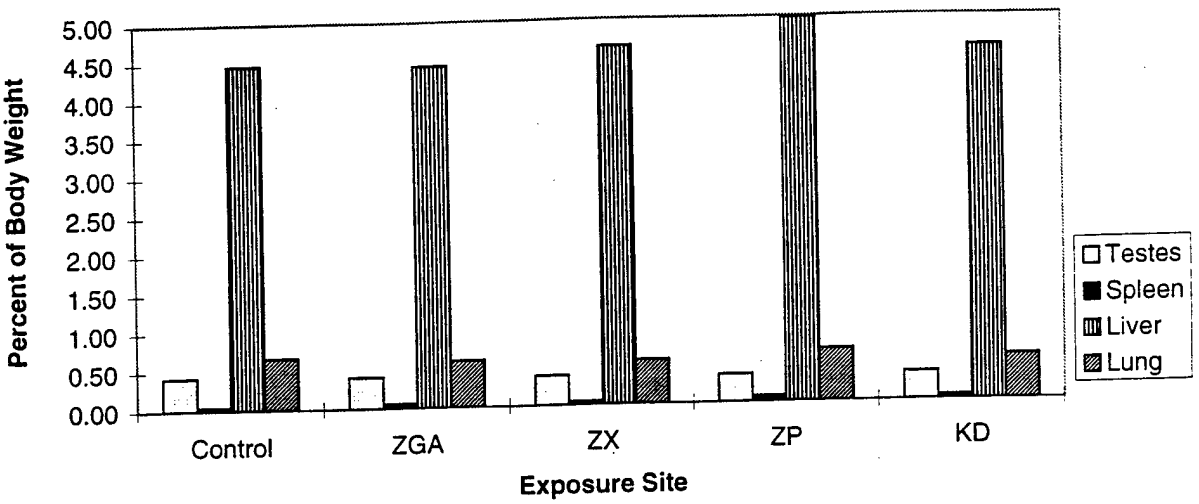


FIGURE 46. SCFE ORAL EXPOSURE #2
PRE REMEDIATION ORGAN WEIGHT DATA

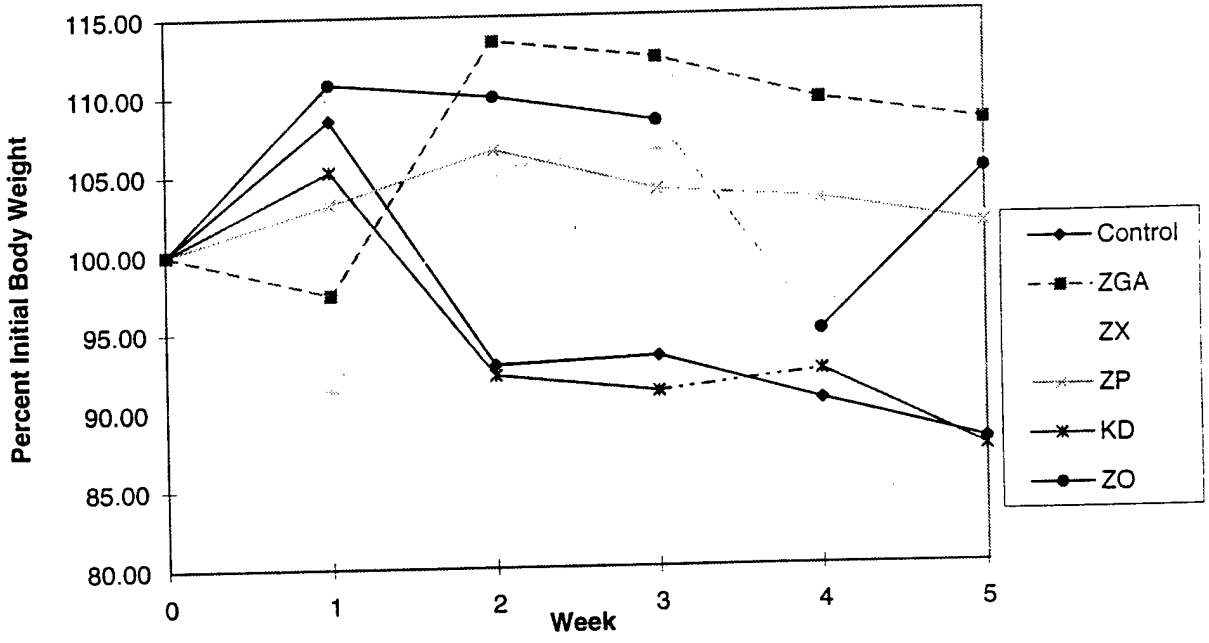


FIGURE 47. DIRECT SOIL EXPOSURE #2
POST REMEDIATION BODY WEIGHT DATA

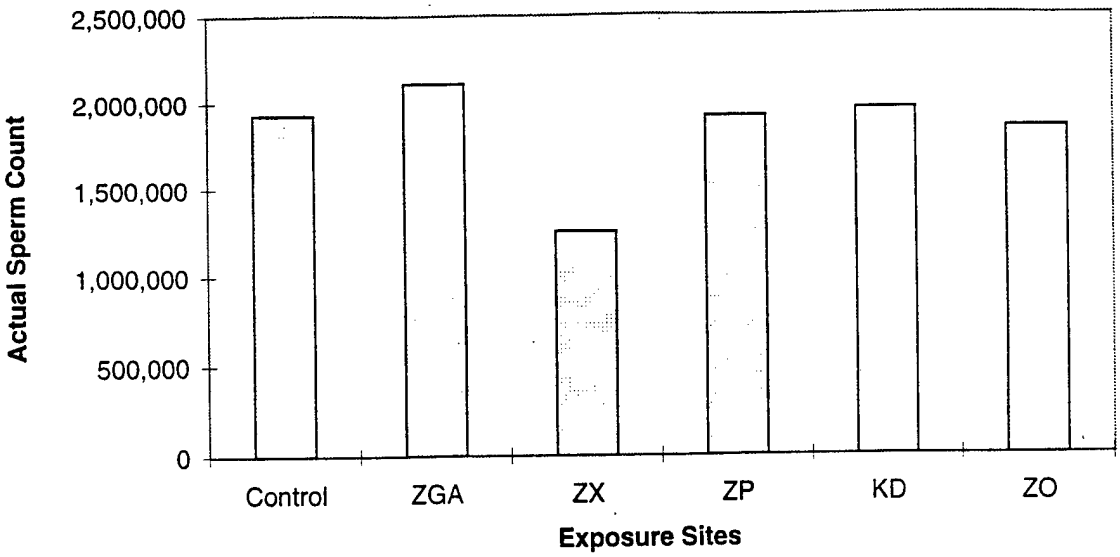


FIGURE 48. DIRECT SOIL EXPOSURE #2
POST REMEDIATION SPERM COUNT DATA

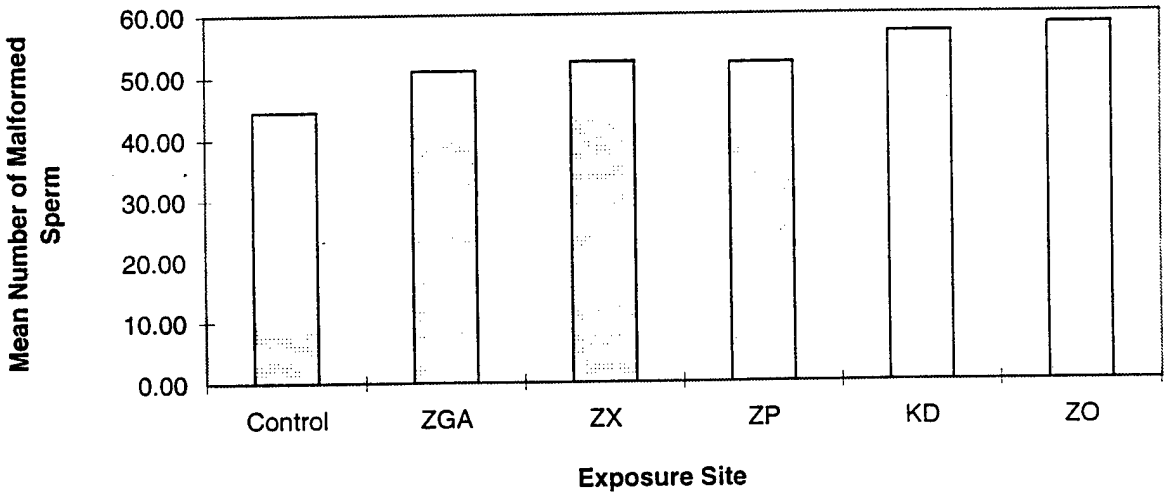


FIGURE 49. DIRECT SOIL EXPOSURE #2
POST REMEDIATION SPERM MALFORMATION DATA

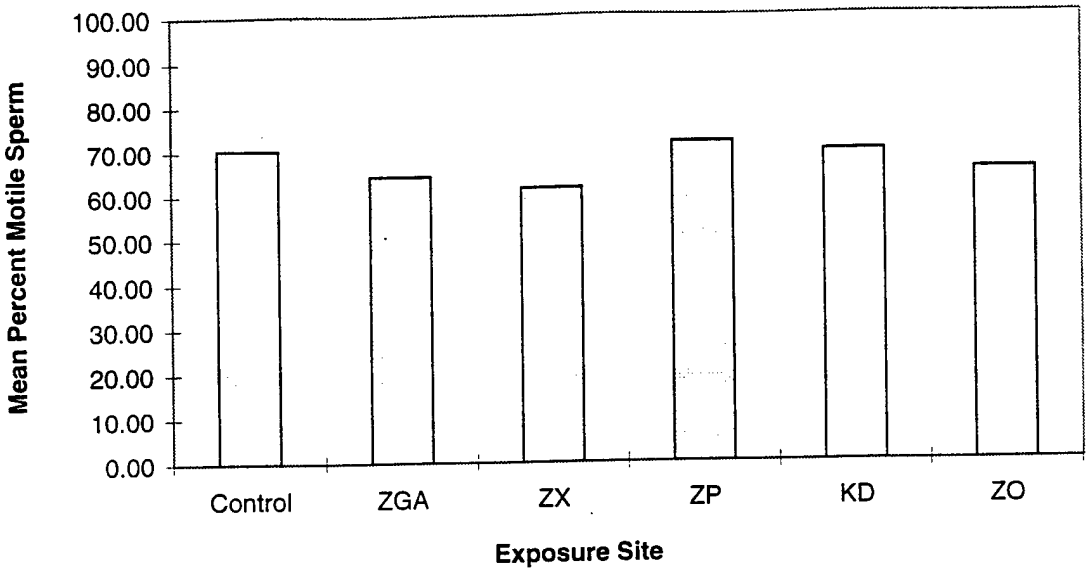


FIGURE 50. DIRECT SOIL EXPOSURE #2 POST REMEDIATION SPERM MOTILITY DATA

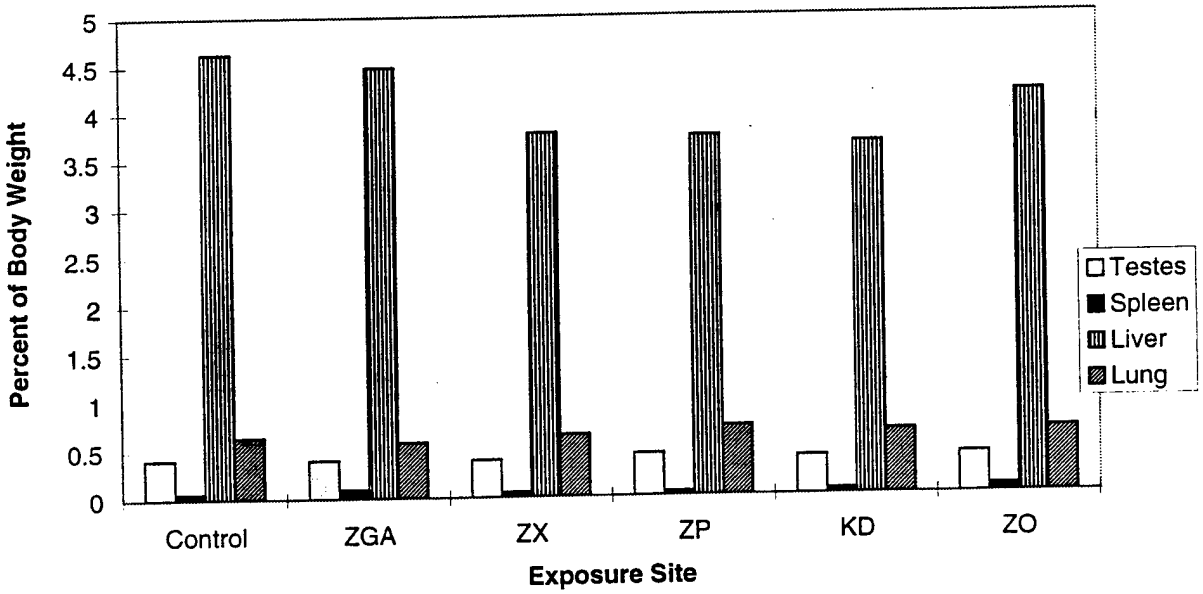


FIGURE 51. DIRECT SOIL EXPOSURE #2 POST REMEDIATION ORGAN WEIGHT DATA

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